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A COMMENT ON THE TIMES

Just as common sense is one of the rarest faculties, so very often what is obvious is not seen. In this case it is the duty of whoever wishes to foster clear thinking to point it out and he may, with luck, easily acquire thorough this a reputation for great wisdom. In medicine there are several instances of this. One such is the truth that when something is adopted only because it is popular at that time and not because it has been shown to be true by scientific observation then those who accept it are inviting disaster. Sometimes even with the greatest care one can be misled such as has been the case in the use of hexachlorophane. The old saying about not being the first by whom the new is tried nor yet the last to lay the old aside has its value, although it could obviously be a dead hand on progress.

When one moves across from the material fields of medicine to those of ethics one is to adopt possibly different criteria. Yet although different these should not be opposite. The point-of-view that there are such things as right and wrong and that these have an objective reality is now curiously unpopular, although this should not be unexpected in times when lunacy (not, regrettably, in the precise legal meaning of the word,

which would at least have the advantage of making its victims "certifiable") is so rampant that the patently abnormal may come to be accepted as normal. Still the fact that this view is unpopular does not make it untrue. "It is not necessarily a condemnation of a man of the past", one wise man said, "to say that his opinions are out of date. There are some dates which it is very good to be out of, and it is most possible that the present is of that type."

It had to be the present to make of work a dirty word, of modesty a curiosity and of pride a virtue. Most glaring of all, gratitude is considered not only an oddity but a sign of weakness and servility. The strong man "who can keep his head when all about him are losing theirs and blaming it on him" will have none of this nonsense and it is the hope that such strong men should arise which can prevent despair. The one point which we would like to dwell upon is the gratitude which the student owes to his teacher because in no other branch of learning so much as in medicine is the relation between master and pupil so close and so vital. It is a glory of medicine that by long tradition the hard won experience of the master is passed on to the apprentice, just as the discovery of the day far from being kept secret is displayed to anyone interested, for comment and discussion. For all this we should be grateful. It is laid down in the Hippocratic Oath, which often sounds surprisingly modern, that the pupil should hold his teacher of medicine equal to his own parents, should make him the partner in his livelihood, should share his own riches with him should he be in need, should consider his family as his own brothers and should teach the members of his family the art of medicine, if they so wish, "without fee or indenture". It is rather a pity that present-day circumstances hardly allow this to be put into practice literally, but it would be good if it were possible for moderns to be given "a chance to acquire merit" as they used to say in "Kim", by practising these virtues. Although this is not practicable along the old lines it is certainly feasible along modern ones with a return to good manners and a gracious respect for one's teachers.

We will add that what is praiseworthy between individuals is even more desirable and admirable between countries. Indeed the reverse of gratitude is a contemptible surliness and arrogance such as is very commonly prevalent now. We have had and have now the repulsive spectacle presented by countries like Uganda, whom the patience and skill of the coloniser lifted out of a brutish and disease-ridden existence into the light of day, not only showing ingratitude but turning upon their teachers. Not from these any thanks for the hard acquired knowledge which a parent country has unstintingly lavished on its charge; not from them the appreciation of what the doctor and the missionary have bestowed at the sacrifice of their lives, of the engineer who has bridged the torrents and overcome distances, of the agricultural expert who has substituted plenty for the erst prevailing hunger, of the teacher and the legislator substituting knowledge and order for ignorance and savagery. It is these same benefactors who are peremptorily being bidden, in the vilest manner, to leave the country they have civilised.

We write of this because medicine is closely concerned with it and it is well that we in Malta should remember and strive to repay the debt we owe to British medicine. Ever since a fortunate historical accident linked our destinies with those of Britain, in countless ways the learning and experience of the Mother Country have been passed on to us without the slightest reserve. This wisdom has, of course, been available to all the world since countries have at least remained sane enough to share such things, but to us it has been given in many special ways: almost every paper in this issue of our periodical bears this out. English, now fortunately accepted as the world language of science, is our heritage and it is something we must cherish and foster in every way. Let us by all means cultivate our own language, but this must never be at the expense of what links us with the vast cultures of Britain and of

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ABNORMAL HAEMOGLOBINS IN MALTA: THE SIGNIFICANCE OF TWO FOETAL AND AN ADULT VARIANT

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The study of human haemoglobin variants has a special place in haemoglobin research for several reasons. Certain amino acid substitutions and deletions in haemoglobin A have contributed significantly to our understanding of the structure and function of the haemoglobin molecule as a cooperative tetramer and further progress in this area can be expected. Foetal haemoglobin variants have shed light on the complicated genetics of the γ-chain of foetal haemoglobin.

The discovery of haemoglobin F (Malta) by Cauchi *et al.* (1969) and the high incidence of this haemoglobin in Maltese newborns gave us the opportunity to initiate a systematic study of a foetal haemoglobin variant. During the course of our screening of cord blood samples we have found a new foetal variant, haemoglobin F-Malta-II. We have also found that some families with Hb-F-Malta-II have a new adult haemoglobin variant, which we have called "Haemoglobin St. Luke's". We have redesignated Hb F(Malta) as Hb-F-Malta-I. The purpose of this article is to give briefly the relevance of Hb-F-Malta-I and Hb-F-Malta-II to current concepts of the genetics of the γ-chain of foetal haemoglobin (Huisman *et al.*, 1972), and to give a brief description of Hb St. Luke's (Bannister *et al.*, 1972).

Foetal haemoglobin variants and the genetics of the γ-chain of foetal haemoglobin

Foetal haemoglobin has two α- and two γ-chains. The γ-chain of human HbF has 146 amino acid residues. Cleavage of the chain at methionyl residues by the action of cyanogen bromide yields three peptides one of which accounts for residues 134-146. Analysis of this peptide called

γCB-3 shows that γ-chains are heterogenous with respect to position 136, which is occupied by two amino acids — glycine and alanine. There are therefore two γ-chains: one with glycine in position 136 and one with alanine in this position. We designate these the Gγ- and the Aγ-chains. The ratio of Gγ- to Aγ-chains at birth is of the order of 2:1 The α-chains of rabbit, horse, goat and mouse haemoglobin are also known to be chemically heterogenous in that certain positions of the polypeptide chains are occupied by more than one amino acid residue. This phenomenon can be explained by invoking the presence of alleles of a single structural gene, by ambiguous translation of genetic material, or by the existence of more than one structural gene. Various considerations support the idea of multiple non-allelic structural human γ-genes (Huisman and Schroeder, 1971). This idea provides a satisfactory mechanistic interpretation of several observations on the γ-chain of human foetal haemoglobin. The original proposal of three Gγ- and one Aγ-genes by Schroeder *et al.* (1968) has been modified to account for recent observations on foetal haemoglobin variants.

It is found that haemoglobin F variants are either mutants of the Gγ- or the Aγ-chain. We consider here four variants which have permitted the formulation of a hypothesis of four non-allelic structural genes for the γ-chain: Hb-F-Malta-I, Hb-F-Malta-II, Hb-F-Jamaica and a Negro Hb-F_x. Hb-F-Malta-I has arginine instead of histidine in position 117 of the γ-chain (Cauchi *et al.*, 1969). We find the incidence of this variant in the Maltese population to be of the order of 2%.Hb-F-Malta-II has a much lower incidence. We have detected six cases of this variant over a period of one year. Paucity of material has so far prevented the elucidation of the amino acid substitution in Hb-F-Malta-II which only contributes a very small fraction to the total foetal haemoglobin when it is present. Hb-F-Jamaica has glutamic acid instead of lysine in position 61 of the γ-chain (Ahern *et al.*, 1970) and we may mention here Hb-F-Hull which has lysine instead of glutamic acid in position 121 (Sacker *et al.*, 1967) and is similar to Hb-F-Jamaica with respect to position 136 and the expression of the γ-chain. Hb-F_x is a Negro variant found in Georgia with a hitherto unknown amino acid substitution.

Hb-F-Malta-I and Hb-F_x are Gγ-variants, while Hb-F-Jamaica and Hb-F-Malta-II are Aγ-variants as shown by analysis of the cyanogen bromide peptide γCB-3 as well as the tryptic peptide γT-15 (residues 133-144) in the case of Hb-F-Malta-I (Cauchi *et al.*, 1969) and Hb-F-Jamaica (Ahern *et al.*, 1970). The normal haemoglobin F in the carriers of these variants shows the same Gγ- to Aγ- ratio as the haemoglobin F of normal newborns.

Haemoglobin F variants also manifest themselves in different, characteristic percentages of the total foetal haemoglobin. We find the percentages of Hb-F-Malta-I, Hb-F_x, Hb-F-Jamaica and Hb-F-Malta-II to be on the average 22.5, 13.5, 12.5 and 5.5, respectively, in heterozygotes. These percentages correspond approximately to 4/18, 2/18, 2/18 and 1/18, respectively, of the total foetal haemoglobin. We have assumed as a working hypothesis that the production of these variants in the heterozygote reflects not only the relative activity of the mutated γ-chain structural gene but also that of the allelic normal γ-chain gene, and accordingly, we have proposed the existence of four non-allelic structural genes for the γ-chain with a relative expression of 4:2:2:1 (Huisman *et al.*, 1972). We designate these presumptive genes $^{G}_{m}\gamma$, $^{G}_{l}\gamma$, $^{A}_{m}\gamma$ and $^{A}_{l}\gamma$, respectively, the symbols G and A referring to the type of γ-chain produced and the symbols m and l indicating the gene with more or lesser activity in each case.

Haemoglobin-F-Malta-I is a $^{G}_{m}\gamma$-variant, Hb-F_x is a $^{G}_{l}\gamma$-variant, Hb-F-Jamaica is a $^{A}_{m}\gamma$-variant, and Hb-F-Malta-II is a $^{A}_{l}\gamma$-variant.

The hypothesis of four γ-chain genes, $^{G}_{m}\gamma$, $^{G}_{l}\gamma$, $^{A}_{m}\gamma$, and $^{A}_{l}\gamma$, with an activity ratio of 4:2:2:1 requires a Gγ- to Aγ-chain ratio of 2:1. This ratio is observed at birth. However, the ratio changes to 2:3 by the fifth month of life and is maintained at this value in the minute amounts of foetal haemoglobin in adult life. It appears that the mechanism which produces the switch from γ-chain to β (and δ)-chain production after birth affects the γ-chain genes differentially. Preferential suppression of the $^{G}_{m}\gamma$-gene might explain the switch from the 2:1 ratio of Gγ- to Aγ-chains at birth to the 2:3 ratio of the adult. We are currently investigating the disappearance rates of Hb-F-Malta-I and Hb-F-Malta-II and the normal haemoglobin F in heterozygous carriers after birth as a way of testing this idea.

Haemoglobin St. Luke's

The haemoglobin molecule is built of two dissimilar sub-units, the α- and β-chains in Hb A which consists of two α- and two β-chains. Haemoglobin A dissociates into dimers containing one α and one β-chain each. The cleavage is believed to occur at the so-called $\alpha_1\beta_2(\alpha_2\beta_1)$ contact which involves 19 amino acids. Oxygenation of haemoglobin results in reorientation of the sub-units. The $\alpha_1\beta_2(\alpha_2\beta_1)$ contacts undergo greater shift than the $\alpha_1\beta_1(\alpha_2\beta_2)$ and assume a new steric orientation. Amino acid substitution in the region of $\alpha_1\beta_2(\alpha_2\beta_1)$ contact may be expected to affect the dissociation and oxygenation properties of haemoglobin. The amino acid substitution in Hb St. Luke's occurs in this region. The haemoglobin is an α-chain variant and in position 95 of the α-chain proline has been replaced by arginine. A comparable situation exists in Hb-G Georgia and Hb-Rampa.. These variants have leucine and serine, respectively, in position 95 of the α-chain (Smith *et al.*, 1972). Like them Hb St. Luke's shows a greatly increased dissociation into dimers in the oxygenated state which seems to be associated with increased affinity for oxygen. The oxygenation properties need further characterization. We have been impeded in this by methaemoglobin formation *in vitro*.

Hb St. Luke's constitutes 10% or less of the total haemoglobin in the cases we have encountered so far. The subjects are healthy and completely asymptomatic. *Table I* gives haematological data on these subjects. We have found Hb St. Luke's in three out of six families with Hb-F-Malta-II. The meaning of the occurrence of both haemoglobin variants in these families is not clear at the present time.

Table I — Haematological data on carriers of the Hb St. Luke's heterozygosity.

Family	*Subject*	*Hb*	*PCV*	*Reticulocytes*	*Hb A*	*Hb A_2*	*Hb St. Luke's*	*Hb A_2 St.Luke's*
		g/100ml	%	%	%	%	%	%
M	*	15.3	47	1.6	87.6	1.6	10.3	0.5
B	P.B.**	16.2	50	1.6	89.4	2.9	7.4	0.3
C	T.C.***	13.9	45	1.3	89.8	2.1	7.9	0.2
G	J.G.****	15.9	46	1.0	90.6	1.9	7.2	0.3

* Average data for four carriers, two males and two females, one being the father of an Hb-F-Malta-II baby.

** Father of newborn baby with Hb St. Luke's heterozygosity detected as 4% Hb-F-St. Luke's.

*** Mother of Hb-F-Malta II baby.

**** Father of Hb-F-Malta II baby.

Acknowledgements

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THE IMMUNOLOGIC DIAGNOSIS OF CANCER

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In view of the explosive nature of the advance in immunology in the last decade, it is inevitable that immunological techniques would be used in an attempt to facilitate the diagnosis of cancer. The purpose of this communication is to put into perspective the various attempts that have been made in this field, and to outline the possible outcome of such research.

The Antigenic Constitution of Human Tumours

Before discussing the possible use of immunological techniques in neoplastic disease it is desirable to outline the ways in which tumour cells differ from normal cells antigenically. As seen in *Table 1*, loss of normal tissue antigens can be an important characteristic of tumours. *Fig. 1* shows a section of human colon stained with an anti-blood group antibody (anti-H) showing that the normal human colonic glands have abundant blood group antigen reactivity while the tumour is depleted of such antigens. Similar loss of histocompatibility, organ specific as well as other normal antigenic constituents have been described. *Fig. 2* shows a section of human colon stained with an anti-colon antiserum. The normal colon stains brightly, while the tumour glands do not.

In contrast to the loss of antigenic constituents by tumour cells described above, it has been shown in recent years that tumours possess antigenic consti-

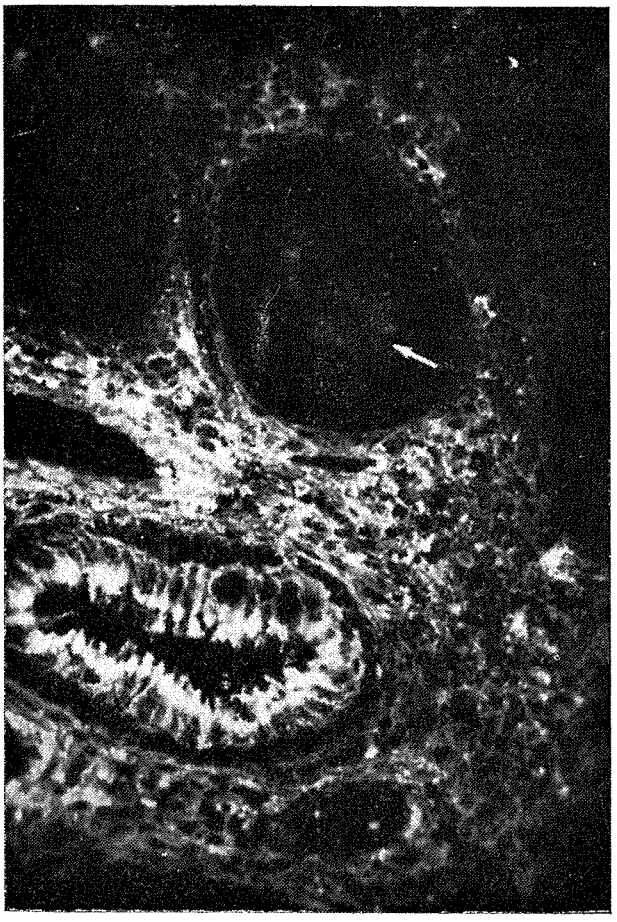

Fig. 1: Section of human colon carcinoma stained with an anti-blood group (anti-H) antiserum. Notice that normal glands stain brilliantly while tumour tissue (arrow) does not. × 210

tuents that are distinct from normal adult antigens. The presence of neoantigens in human tumours has now been adequately established in conditions such as Burkitt's lymphoma (Klein *et al.*, 1967); melanoma (Lewis *et al.*, 1969), neuroblastoma (Hellstrom *et al.*, 1968); osteogenic sarcoma (Morton and Malgren, 1968); bladder tumours (Bubenik *et al.*, 1970); Hodgkin's disease (Order *et al.*, 1971) and leukaemia (Mathe *et al.*, 1969).

In the serum of human foetus one can portant recent results on the identification and practical application of immune responses to the diagnosis of human tumours will be delineated.

Table 1

Antigenic Changes in Tumours

1. *Loss of Normal Antigens*, e.g.
   a. blood group antigen
   b. organ specific antigen
   c. histocompatibility antigen.
2. *Reversion to Foetal Type Antigenic Constitution*, e.g.
   a. Carcinoembryonic antigen
   b. α-foetoprotein production
   c. metaplasia-associated antigen.
3. *Gain of Tumour Specific Antigens*
   a. individual specific antigens, e.g. carcinogen induced.
   b. group antigen (cross-reacting) e.g. viral induced tumours, Burkitt's lymphoma, melanomas, carcinoma of the colon, etc.

α-Foetoprotein and Hepatoma

In the following pages the more imdetect by gel diffusion and immunoelectrophoresis techniques a globulin which migrates in the α_1 region *(Fig. 3)*. Such a globulin is not detected in normal adult sera. In 1964 Tatarinov reported the presence of this foetoprotein in the sera of patients with primary hepatoma. *Fig. 4* shows the gel diffusion pattern of foetal serum and serum from patients with hepatoma. This is a simple test for hepatoma, being positive in 50-80% of patients who have it. This test is being used at the moment in a WHO survey of the incidence of hepatoma in Africa (Muir, C.S., personal communication). The recent demonstration of minute amounts of foetoprotein by radioimmunoassay techniques in normal adult sera (Rouslahti and Seppala, 1971) does not detract from the practical usefulness of this test in clinical practice.

The significance of a foetoprotein reemerging in the adult circulation is a very intriguing one. Using tissue culture techniques Gitlin and Boesman (1967) have shown that the protein can be produced by foetal liver cells. We have localised

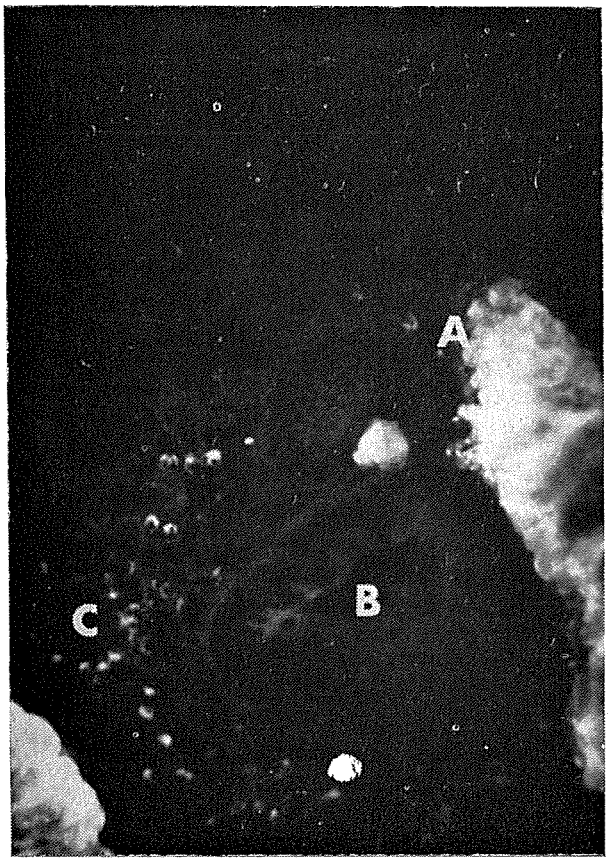

Fig. 2: Section of human colon carcinoma stained with a specific anti-colon antiserum to show absence of staining in the tumour glands (B) compared to normal (A), indicating loss of organ-specific antigens. × 210

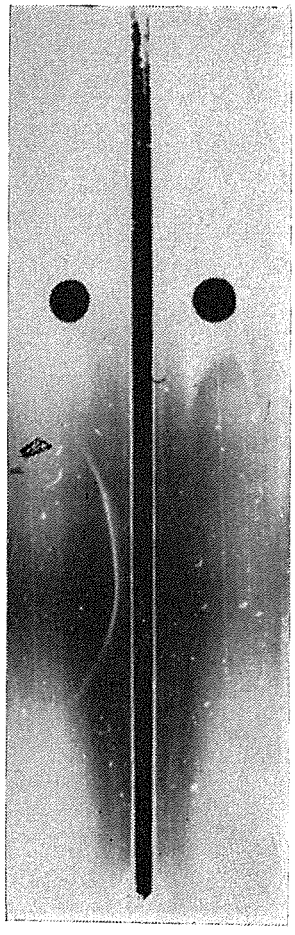

Fig. 3: Immunoelectrophoresis of human foetal serum reacted with an antifoetoprotein antiserum: α-foetoprotein migrates in the α_1 globulin region

α-foetoprotein in human foetal liver cells and hepatoma using indirect immunofluorescent techniques (Cauchi and Nairn, 1972a). Briefly, fresh tissues were snap frozen in a liquid nitrogen-isopentane mixture and 6μ thick sections were cut, stained with a specific rabbit anti-α-foetoprotein, washed and a fluorescein-conjugated goat-anti-rabbit globulin was added. Using this technique, the presence of cells which are capable of binding with an anti-α-foetoprotein anti--serum, (and hence presumably containing α-foetoprotein) could be demonstrated in both foetal liver *(Fig. 5)* as well as in sections from a hepatoma, but not in normal tissues.

Testing for α-foetoprotein has now become a routine test in a large number of hospital laboratories and has proven to be the most simple specific test for human hepatoma. The test is indicated in patients with:

a. a hepatic mass of unknown aetiology,

b. cirrhosis or chronic liver disease of any type,

c. presumed "metastatic" cancer where a primary tumour cannot be detected (Stillman and Zamcheck, 1970).

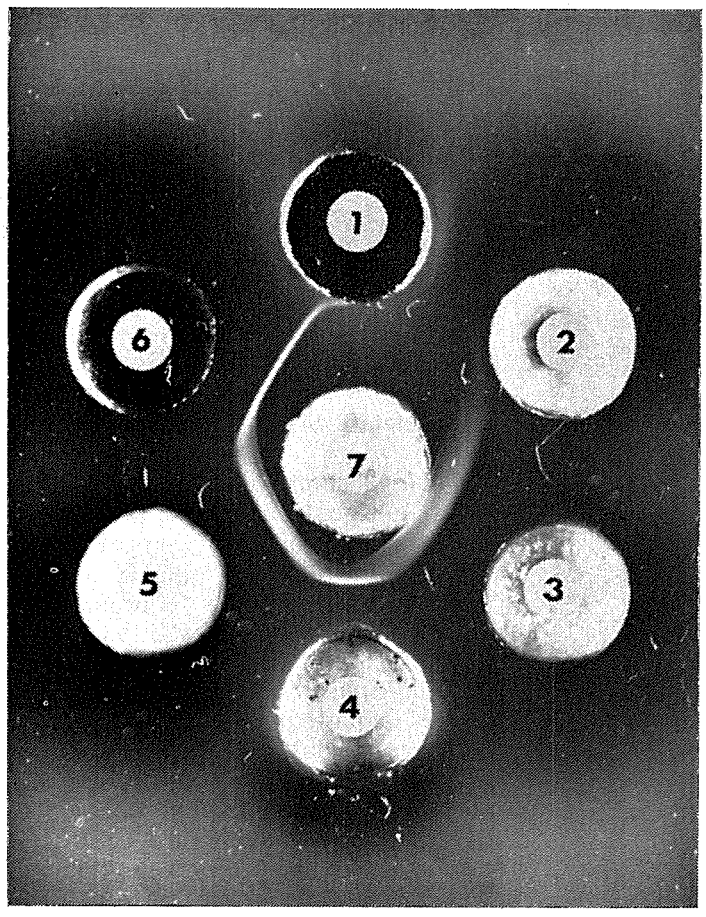

Fig. 4: Gel diffusion showing immunological reaction between a specific anti-foetal serum (centre well) and human foetal serum (wells 3 and 6), amniotic fluid (well 4), and serum from a patient with hepatoma (well 5). There is no reaction with normal adult serum (well 2).

Carcinoembryonic Antigen (CEA) and Gastrointestinal Cancer

In 1965, Gold and Freedman obtained an extract from carcinoma of the colon and succeeded in immunizing rabbits to produce an antibody which reacted specifically with colonic cancer but not with normal tissue. Later on this antigen was also found in other tumours of entodermal origin, e.g. oesophagus, stomach, pancreas, as well as in metastases of such tumours in the liver. As this antigen was shown also to be present in the normal gastrointestinal tract of human foetuses of 2-6 months gestation, this antigen was called carcinoembryonic antigen (CEA).

The real impetus to the clinical application of this knowledge came with the discovery that CEA is present in detectable concentrations in the sera of patients with cancer of the gastrointestinal tract. Using a sensitive radioimmunoassay technique capable of detecting 2.5ng of CEA/ml (1 ng = 10^{-9} gm) Gold and his associates (Thompson *et al.*, 1969) have shown that the level of CEA can be higher than 300 ng/ml in some patients with carcinoma of the colon. 97% of patients with carcinoma of the colon had significantly higher than normal levels of CEA in the serum.

Since these original discoveries a number of tumour immunologists have been engaged in the production of a simple and reliable immunoassay test for the detection of carcinoma of the colon. At the moment we are in the process of providing a simple radiometric test that can be

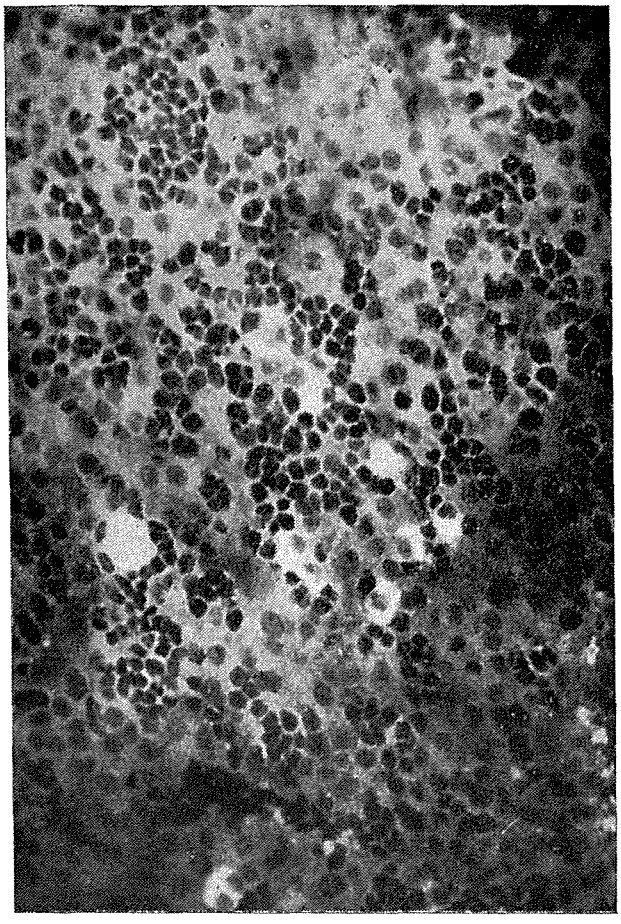

Fig. 5: Frozen section of foetal human liver stained with anti-foetal serum showing fluorescence of hepatic cells × 210

applied to the detection of CEA (Reid and Cauchi, 1972).

The presence of *antibody* specific to CEA in the serum of some patients with carcinoma of the colon has been described (Gold, 1967) but data on this topic are conflicting (Lo Gerfo *et al.*, 1972). It is conceivable that a large tumour mass can act as an antigenic "sink" absorbing all serum antibody activity. It is more likely, however, that the host is in some way incapable of responding to the large paralysing dose of circulating antigen, giving rise to a situation analogous to "immunological paralysis" (Mitchison, 1964). It is unlikely that the host is inherently incapable of responding to foetal-type antigen if these were presented in a suitable manner, since we have shown that a host can produce specific anti-foetal antibody activity when immunized with foetal tissues (Cauchi and Nairn, 1972b). *Fig. 6* shows a foetal intestinal cell stained by a specific isologous antifoetal intestine antiserum, showing the typical membrane staining. As illustrated in this figure, CEA is largely a surface bound antigen.

Other "Embryonic" type Antigens Associated with Neoplastic Disease

Table 2 summarises the data on the various antigenic systems described in human tumours relating to embryonic type antigens. The placental alkaline phosphatase (Regan isoenzyme) is found in the sera of 4-5% of patients with various malignancies. Stohlbach *et al.* (1969) found an alkaline phosphatase isoenzyme in the serum of a patient (Regan by name) but not in normal adult tissues. It is chemically and immunologically indistinguishable from placental alkaline phosphatase. It appears to be produced by the tumour itself. The heterophile foetal antigen described by Edynak *et al.* (1970) is non-species-specific and seems to be present in a large variety of tumours, benign or malignant. To date these tests have not found widespread clinical application, but might be useful eventually as non-specific tests for the detection of cancer.

Metaplasia-associated Antigen

It has been shown that intestinal metaplasia which occurs in gastric tumours in man (Ming *et al.*, 1967) and animals (Feit *et al.*, 1967) may be associated with the loss of adult-specific antigens and the re-emergence of an intestinal-type antigen (de Boer *et al.*, 1969; de Boer and Cauchi, 1971). This can be shown quite readily in the experimental animal following such carcinogenic agents as X-irradiation. Such a distribution of intestinal antigen in the stomach is incidentally the normal finding

in the foetus (de Boer *et al.*, 1969), indicating that this antigenic change can be considered as a reversion to a foetal antigenic distribution. *Fig.* 7 shows a frozen section of human carcinoma stained with an antiserum to show intestinal-type antigen.

The search for antigenc constituents in gastrointestinal secretions which are specific for cancer appears attractive. Hakkinen and Viikari (1969) reported a new test for the diagnosis of gastric cancer based on the detection of a sulphoglycoprotein in the gastric juice of patients with gastric cancer. This test was positive in 96% of patients with gastric cancer compared to 9.4% of "control" patients. The relatively high incidence of false positive tests in conditions like benign peptic ulcer (14%) limits the usefulness of such a test until it can be demonstrated that the test can be made more specific for gastric neoplasm.

Tumour Antigens in Lymphoma and Leukaemia

In 1964, Epstein *et al.*, found intracellular DNA virus, now known as the Epstein Barr Virus (EBV) in cultured cells from a patient with Burkitt's lymphoma. Antibodies to EBV were found in a large proportion of normal people tested, and their distribution is world wide. However, high levels of anti-EBV antibody titres (more than 1:160) have been found only in three conditions viz: infectious mononucleosis, Burkitt's lymphoma and nasopharyngeal carcinoma (Henle and Henle, 1969). Klein *et al.*, (1966) using indirect immunofluorescent techniques demonstrated 7S immunoglobulins in the sera of patients with Burkitt's lymphoma that reacted with surface antigens of lymphoma cells, but not of normal cells. Other antibodies reacting with cytoplasmic constituents of

Table 2

Embryonic-Type Antigenic Constituents in Human Tumours

Antigen	*Cancer*	*Localisation*	*Ref.*
α-foetoprotein	Hepatoma, teratoma	serum, tumour	Abelev *et al.* 1967
Carcinoembryonic (CEA)	GIT cancer	cell membrane (glycocalix) serum	Gold and Freedman 1965
Foetal Sulphoglycoprotein	Gastric cancer	gastric juice	Hakkinen 1966
Tumour glycolipids	GIT cancer	tumour	Rapport *et al.* 1959
T-globulin	Various cancers	serum	Tal *et al.* 1970
Regan isoenzyme	Digestive tract and various malignant tumours	tumour cells	Stolbach **et al.** 1969
α_2-ferroprotein	Malignant tumours of childhood (nephroblastoma, neuroblastoma, teratoma, etc.)	serum	Buffe *et al.* 1970
Heterophile foetal Antigen	Various tumours	serum	Edynak *et al.* 1970

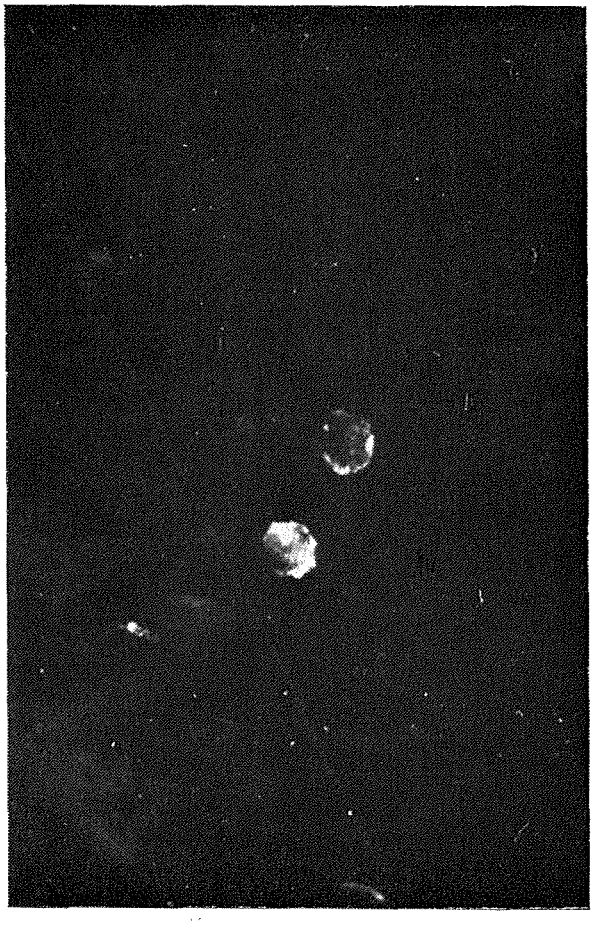

Fig. 6: A foetal intestinal cell stained by an anti-foetal serum to illustrate cell membrane immunofluorescence × 540

lymphoma cells have been described by Henle *et al.*, (1969). The following antigens have now been described in Burkitt's lymphoma cells (Clifford, 1972).

1. *Early antigen* (EA): a soluble antigen which appears intracellularly soon after infection with EBV.
2. *Membrane antigen complex* (MA): on the surface of Burkitt's lymphoma cells, probably representing viral envelope constituents.
3. *Viral capsid antigen* (VCA): a "late" viral product present in the cytoplasma of Burkitt's lymphoma cells.

Although such antigenic systems have not been used for the diagnosis of Burkitt's lymphoma, further studies might indicate that changes in the levels of antibody might be related to prognosis following surgery, chemotherapy etc. For example, the highest levels of anti-VCA titres were found in patients with large tumours, while low levels were found in patients with long term remission. On the other hand, high anti-MA titres correlated better with a small tumour mass, indicating absorption of the antibody by the tumour mass (Henle *et al.*, 1969). A rise in anti-MA antibody titres usually occurs in patients who respond well to chemotherapy or local irradiation (Einhorn *et al.*, 1970).

During the last few years we have been working on the antigenic constitution of leukaemic cells and their ability to stimulate an immune response in the patient. There is little doubt now that leukaemia-specific antigens do exist (Viza *et al.*, 1969) and that the patient can respond to such antigens by the production of antibodies or a cell-mediated immune response (Powles *et al.*, 1971). More work is, however, required before these data can be of prognostic value. In particular with the more widespread use of immunotherapeutic techniques, the accurate measurement of the immune status will be an essential parameter in detecting host response. That such a response is necessarily complex, time — as well as dose — related has been shown extensively in experimental systems (Cauchi, 1972).

Immunologic Detection of Tumour Secretions

Certain tumours secrete substances or hormones which can be detected by sensitive immunological techniques. For example, radioimmunoassay techniques have been used to measure chorionic gonadotrophin from chorionepitheliomas, calcitonin from thyroid carcinoma, gastrin from pancreatic tumours producing the Zollinger Ellison syndrome, insulin from pancreatic tumours, and ACTH from Pituitary and lung tumours (WHO, 1972). These methods may eventually become useful in

Fig. 7: Section of irradiated stomach stained with an anti-colon antiserum to show the presence of intestinal-type antigens × 340

the diagnosis of hormone-secreting tumours.

Macrophage Slowing Factor (MSF) in Cancer

It has been shown recently that blood lymphocytes from patients with neoplastic disease were sensitized to an antigen which could be extracted from normal brain (Field and Caspary, 1970), or from a variety of malignant tumours (Caspary and Field, 1971). Following interaction of lymphocytes with the antigen, a factor is released which slows the migration of macrophages by about 14-20% of normal. This is a relatively simple test which might eventually be useful as a non-specific test for cancer.

Autoantibodies in Neoplastic Disease

It is very difficult to interpret the significance of autoantibodies to normal tissue constituents in patients with neoplastic disease. Firstly some forms of neoplastic disease have a greater tendency to occur in patients with specific autoimmune diseases. For example carcinoma of the colon is about 20 times more common in patients with ulcerative colitis than in the general population. Secondly, tumours and autoimmune disease process might be associated in some ill defined way, e.g. the presence of thymoma and myasthenia gravis. Thirdly, there is the well known association between autoimmune haemolytic anaemia and conditions like Hodgkin's disease, lymphatic leukaemia and more recently other tumours, e.g. ovarian dermoid cyst (Baker *et al.*, 1968). Fourthly, and perhaps more specifically, tumours might stimulate the production of antibodies that cross react with normal constituents. The finding of a smooth muscle antibody in 67.5% of patients with malignant disease (Whitehouse and Holborow, 1971) falls in this category. This work needs confirmation. We have not been able to confirm such findings (Tannenberg *et al.*, 1972).

The destruction of a large mass of tissue by irradiation, chemotherapy etc., might itself produce sufficient stimulus for the production of autoantibodies. Antibody formation against skin (Quismorio, *et al.*, 1971), leucocytes (Price *et al.*, 1969), nuclei (Weir, 1966), etc. have been shown to occur after tissue necrosis following heat, radiotherapy, or infarction.

Although interesting biologically, such autoimmune phenomena are unlikely to be of practical value in the diagnosis of cancer.

Conclusions

One can envisage a number of ways in which immunologic tests for cancer can be useful in future clinical practice.

a. In the diagnosis of tumours prior to surgical biopsy. So far this is a routine test only in the case of hepatocellular carcinoma.

b. In the detection of recurrence after surgical removal of a primary carcinoma. This has obvious application in gastrointestinal tract cancer, hepatoma, etc.

c. In screening programmes for the detection of cancer where there is an endemically high incidence, e.g. hepatoma, Burkitt's lymphoma in Africa.

d. In determining the prognosis of tumour patients. It has been shown that antibody titres vary according to tumour mass, the presence of metastasis etc. This may be correlated with ultimate prognosis.

e. Using radioactively labelled highly specific antitumour antibodies, the localisation of tumour metastasis, involved lymph nodes, etc. would provide a rapid objective assessment of tumour spread.

f. As an objective test in the control of tumours by immunotherapy.

In painting an optimistic future for the role of tumour immunology in clinical practice one must not forget that a great deal of fundamental work is still required to delineate the exact biological significance of tumour-specific antigens. Such work at the moment is being carried out in major tumour immunology centres throughout the world, and one would hope that the next ten years will witness the same rate of growth in the application of immunological techniques to the cancer problem that has been characteristic of basic immunological research in the last decade.

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LUMBAR SYMPATHECTOMY IN THE TREATMENT OF INTERMITTENT CLAUDICATION: AN OBJECTIVE STUDY

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Summary

Sympathectomy in twenty legs suffering from atherosclerotic peripheral vascular disease made no improvement in the muscle blood flow as detected objectively by the Technetium (^{99m}Tc) Clearance Technique in nineteen of these legs. It is postulated that intermittent claudicaion *per se* is not an indication for sympathectomy although the operation may have a place in the treatment of early nutritional disturbances in the ischaemic leg.

It is still a fairly widespread practice to submit patients who present with the symptoms of intermittent claudication to the operation of lumbar sympathectomy. It is the impression of some surgeons that many of these cases acquire worthwhile improvement in their symptoms. However, there is no objective evidence to support this view and indeed there is experimental evidence (Myers and Irvine, 1966a) that the operation should not be expected to allay the pain of intermittent claudication.

The object of this paper is to present the results of an objective study on a series of patients, all complaining of intermittent claudication (some also with rest pain and/or trophic skin changes) who were assessed both clinically and objectively using a radio-active tracer technique before and after they had undergone lumbar sympathectomy.

Material

Fifteen patients, all with occlusive peripheral arterial disease proved by arteriography, were studied. Five patients had symptoms in both legs so that, in all, this series comprises twenty limbs. All patients complained of intermittent claudication in one or both limbs, four patients (six legs) had rest pain while marked ischaemic (trophic) changes were present in ten legs.

Method

All legs were studied by recording muscle blood flow in the lateral gastrocnemius at rest and during exercise. The technique has been described elsewhere (Cutajar *et al.* 1971).

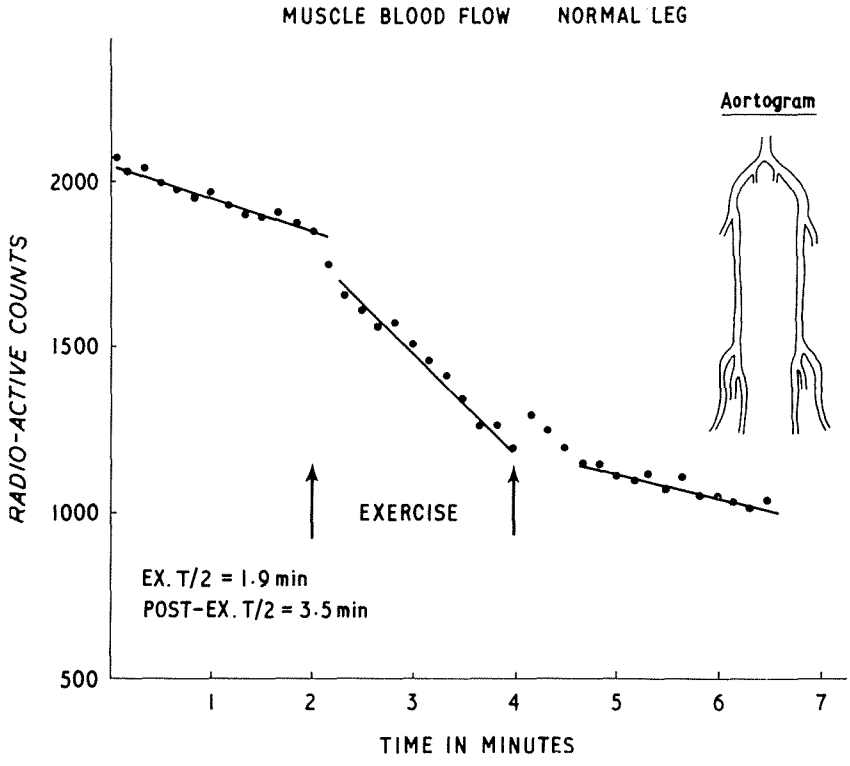
MUSCLE BLOOD FLOW NORMAL LEG
Aortogram
RADIO-ACTIVE COUNTS
2000
1500
1000
500
EXERCISE
EX. T/2 = 1.9 min
POST-EX. T/2 = 3.5 min
1
2
3
4
5
6
7
TIME IN MINUTES

Fig. 1

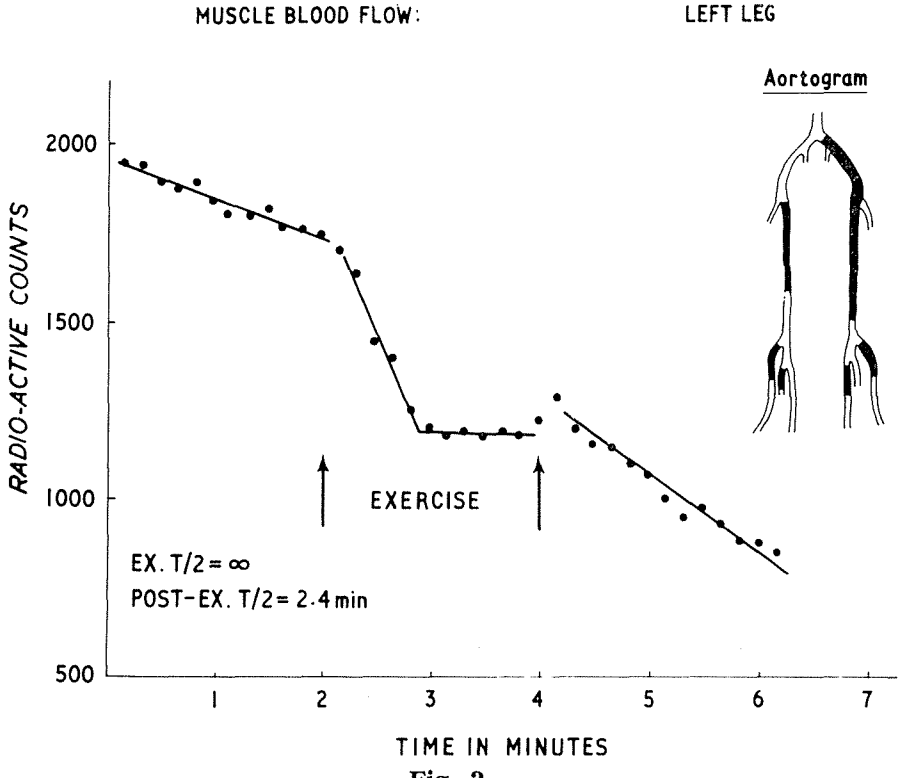
MUSCLE BLOOD FLOW:
LEFT LEG
Aortogram
RADIO-ACTIVE COUNTS
2000
1500
1000
500
EXERCISE
EX. T/2 = ∞
POST-EX. T/2 = 2.4 min
1
2
3
4
5
6
7
TIME IN MINUTES

Fig. 2

Briefly, the technique consists in injecting 100 μC of the radio-active isotope, TECHNETIUM (^{99m}Tc into the lateral gastrocnemius of the leg under observation. The clearance of the isotope is measured by means of a Geiger counter tube strapped directly over the site of the injection (fig. 1), the count-rate being recorded by an electronic rate-meter which is connected to a chart recorder which automatically plots the count rate against time thus giving a clearance curve. The experiment is conducted under various physiological conditions, i.e. with the patient at rest, during exercise and in the post-exercise period.

Fig. 2 shows a typical clearance curve in a leg with normal circulation. In the initial resting period there is a steady but comparatively slow clearance of ^{99m}Tc from the muscle. With the onset of exercise there is a sharp increase in clearance (and therefore in the blood flow) which persists throughout the whole exercise period. In the post-exercise period there is again a diminution in clearance, which gradually returns to the resting level.

In contrast fig. 3 shows what happens in a limb which is the site of an arterial occlusion due to atherosclerotic peripheral vascular disease. Whereas the clearance of ^{99m}Tc (and hence the blood flow) in the pre-exercise resting period is similar to that of the normal limb, there is considerable diminution in clearance in the claudicating limb during the period of exercise.

These clearance patterns were found to be so constant that it was possible to assess objectively the result of any form of surgery in a claudicating limb. For example, an abnormal muscle blood flow clearance curve was converted to a normal one following successful arterial reconstruction.

Results

In the present series, pre- and post-operative muscle blood flow curves were obtained in twenty limbs sympathectomised for peripheral arterial occlusion.

TABLE 1
Symptoms in 20 legs with P.V.D.

Int. claudication: 14.
Rest pain: 6.
Marked trophic changes: 10.

Table 1 describes the symptoms of the limbs under observation. All these limbs had an abnormal muscle blood-flow clearance (^{99m}Tc) curve prior to sympathectomy.

Post-operatively patients were questioned regarding their symptoms and all legs were studied objectively by repeating

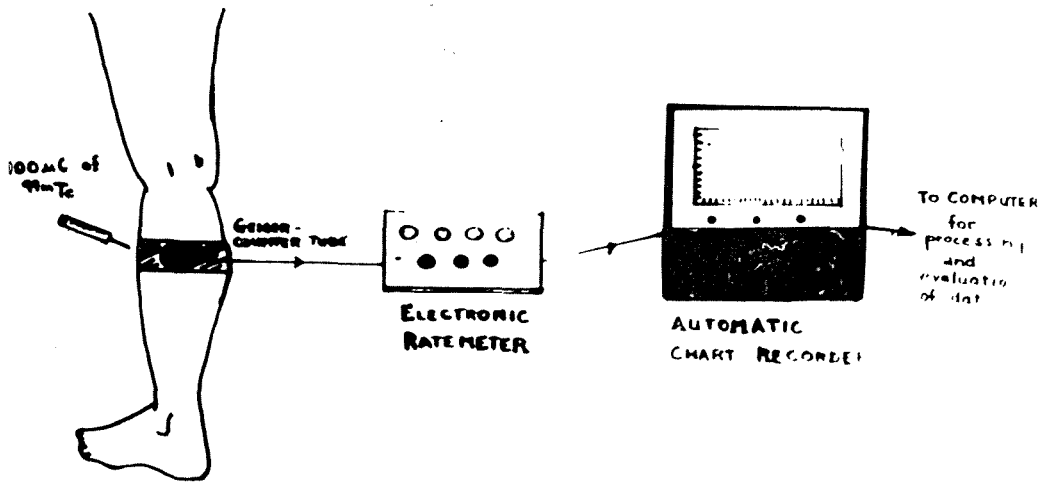

Fig. 3: Muscle blood flow studies by the Technetium (^{99m}Tc) clearance technique.

the clearance test. Table 2 shows the results of sympathectomy in these legs. It is obvious that although many patients felt that they had improved after the operation there was in fact no change in the actual muscle blood flow (as tested by the Technetium clearance technique) in the majority of the operated limbs.

TABLE 2

No. of Legs: 20 (15 patients).
Subjective improvement after Sympathectomy:
"Improved": 10 legs (inc. 4 with rest pain).
"Same": 6 legs.
"Worse": 4 legs.
Objective Change: only 1 leg showed a change in ^{99m}Tc Clearance to a normal pattern.

Discussion

The results obtained in this study seem to indicate that sympathectomy cannot be relied upon to augment blood-flow to ischaemic muscle to any important degree and that any improvement which there may have been in operated patients was more psychological than actual. This was also the conclusion of Myers and Irvine (1966a) and of Taylor and Calo (1962).

Consideration of the sympathetic innervation of the muscle circulation lends little support to the rationale of denervation. The nervous control of the muscle vessels is much less pronounced than for the skin circulation. At rest vasomotor tone in muscle is mainly determined by inherent myogenic tone in the arteriolar wall and under normal conditions the effect of the sympathetic vasoconstrictor innervation is minimal. Stimulation of sympathetic vasodilator fibres may increase muscle flow but only under situations of marked stress.

In contrast to the muscle circulation, there is a high level of sympathetic vasoconstrictor tone of skin vessels in the foot, and their sympathetic denervation initially results in marked vasodilatation (Myers and Irvine, 1966b). In the present series it was noted that there was often an improvement in early ischaemic cutaneous lesions of the affected leg and also of mild rest pain although the claudication pain was largely unaffected.

Acknowledgements

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STUDIES ON THE ROLE OF COMPLEMENT IN THE NEUTRALIZATION OF HERPES SIMPLEX VIRUS

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(Abridged from a report on the results of researches undertaken during studies for the degree of Master of Science in the Department of Virology of the University of Birmingham, September 1972).

Summary

Complement was shown to enhance the neutralization of herpes simplex virus. Early and late rabbit anti-herpes sera neutralized virus in the absence of complement, although neutralization was enhanced on the addition of complement. In the literature, these types of antibodies have been classed as non-complement requiring neutralising antibodies (non-CRN antibodies).

Preliminary investigation with early (acute) human anti-herpes serum showed low or negligible neutralization in the absence of complement which was greatly enhanced when complement was added. The late (convalescent) human anti-herpes serum neutralized virus in the absence of complement although again, enhancement occurred when complement was present. Complement-requiring neutralizing antibodies (CRN antibodies) were thus the main types of antibodies present in the early phases after infection with herpes virus and these were later replaced, as the infection progressed, by non-CRN antibodies.

Attempts were made to demonstrate the types and time of appearance of CRN and non-CRN antibodies in mice after immunization with herpes virus. Early mouse anti-herpes serum showed low neutralizing activity in the absence of complement which was only slightly enhanced by the addition of complement. Later sera appeared to contain antibodies of the non-CRN type since neutralization was marked in the absence of complement.

Introduction

It has been known for some time that a factor present in normal serum enhances the action of neutralizing antibodies against various types of viruses. Gordon (1925) working with vaccinia virus showed that heating lowered the neutralizing potency of immune serum. The enhancing effect of a heat-labile substance on the neutralizing activity of immune serum was demonstrated for Rous sarcoma virus (Muller, 1931), Western equine encephalitis virus (Morgan, 1945; Whitman, 1947), mumps virus (Leymaster and Ward, 1949), Newcastle disease virus (Howitt, 1950), dengue fever virus (Sabin, 1950) and variola virus (McCarty and Germer, 1952). Dozois *et al.* (1949) showed that the nature of the heat-labile substance responsible for the enhancing effect on anti-WEE neutralizing antibodies were the second, third and fourth components of complement. The role of complement as the enhancing factor in virus neutralization was firmly established by similar studies with herpes simplex virus (Taniguchi and Yoshino, 1965).

Early and late immune sera differ in their requirement for complement for subsequent neutralization of virus. Thus, studies with early sera obtained from rabbits immunized with herpes simplex virus

showed that these mainly contained CRN antibodies while late sera were non-complement requiring, although complement also enhanced the neutralizing activity of such late antisera (Yoshino and Taniguchi, 1964; Wallis and Melnick, 1971). Similar results were obtained with guinea pig anti-herpes sera (Yoshino and Taniguchi, 1966). Adler *et al.* (1971) showed that complement amplifies the activity of rabbit anticoliphage early IgM and IgG antibodies up to a thousandfold.

CRN antibodies were also found in the early sera of rabbits artificially infected with herpes virus and in naturally infected man (Yoshino and Taniguchi, 1964; 1966). In cases of herpes virus infections, serum taken early in the disease was shown to neutralize virus only in the presence of complement. This finding has considerable diagnostic value as the presence of CRN antibodies can be taken as evidence of infection. The finding of CRN antibodies in early immune sera thus appears to be a common phenomenon since they have been detected in various species of either immunized or infected animals including man.

This thesis examines the role of complement in the neutralization of herpes simplex virus by early and late antisera raised in rabbits by an immunization scheme which differs from that employed by several authors (Yoshino and Taniguchi, 1964; Hampar *et al.*, 1968; Wallis and Melnick, 1971). The resulting differences obtained, especially with the early sera, will be analysed and discussed. The results obtained with the human acute and convalescent anti-herpes sera were similar to those obtained by Yoshino and Taniguchi (1966).

In order to investigate further the time of appearance of CRN and non-CRN antibodies, mice were immunized with herpes simplex virus and the immune sera obtained at various time intervals were tested in neutralization experiments in the presence and absence of complement. The results were in many respects similar to those obtained by Yoshino and Taniguchi (1964) who used rabbits in their experiments.

Materials and Methods

Cultured cells

BHK21 (baby hamster kidney) cells (Macpherson and Stoker, 1961) were used throughout this study.

Growth medium and diluents

Cells were serially subcultured and maintained in modified Eagle's medium (Vantsis and Wildy, 1962) containing 10% calf serum and 10% tryptose phosphate broth (ETC). Stock virus suspensions were prepared in ETC and, when diluted, the same medium was used as diluent. All sera and complement, whether unheated or inactivated, were diluted in Dulbecco A solution (0.8% NaCl, 0.02% KCl in phosphate buffer, pH 7.0) (Dulbecco and Vogt, 1954).

Virus production

The HFEM strain was used throughout as herpes simplex virus type 1. This strain was also used to immunize rabbits and mice. Herpes simplex virus type 2 (strain 3345) was used in some of the neutralization tests. Both virus types were grown in BHK21 cells. The cells were first allowed to form confluent monolayers in 80 oz. Winchester bottles after which each was infected with 10^7 plaque forming units (p.f.u.) of virus in 20 ml ETC. The virus was allowed to adsorb for 60 min at 32°C and then a further 180 ml of ETC was added and incubation continued for 2 to 3 days. Widespread cytopathic effect was usually observed by this time. After decanting most of the medium, the cells were scraped off into a small volume of remaining ETC and then centrifuged at 1000 rev/min for 10 min. The sedimented cells were resuspended in 5 ml fresh ETC and disrupted with an ultrasonic probe (MSE Ltd., Crawley, Sussex). The disrupted material was then centrifuged at 4000 rev/min for 15 min, the supernatant fluid collected and stored in small volumes at -70°C until used.

Virus assays

Assays were performed by the suspension-plaque method of Russell (1962). Briefly, 2 ml of appropriate virus dilutions were incubated with 7 × 10^6 BHK cells for 60 min at 37°C on a shaker. Then, 8 ml of 0.8% carboxymethyl cellulose (CMC) in ETC (CMC/ETC), prewarmed to 37°C, were added and the resulting suspension poured into two 45 mm plastic plates which were subsequently incubated at 37°C in a 5% CO_2-air incubator. At the end of the incubation period (usually 2 days), the medium was decanted and the monolayers washed once with phosphate buffered saline. They were then fixed for 10 min with 10% formol saline. After removal of the fixative, the plates were washed in tap water and then stained with dilute carbol fuchsin for 10 min followed by washing twice in tap water. Plaques were counted with a plate microscope using a × 2.5 magnification.

Neutralization tests

Kinetic neutralization experiments were carried out throughout this study. Unless otherwise stated, the following volumes of reagents and virus titres were used in these tests.

0.05 ml of an appropriate dilution of immune serum was mixed with an equal volume of guinea pig complement diluted to contain 10 units in 0.05 ml volume. 0.1 ml of a suspension of virus containing 5 × 10^4 p.f.u./ml was then added and the mixture left to incubate at room temperature for the time indicated in the Figures. Then 0.1 ml was diluted in 9.9 ml ETC and residual infectivity was assayed as described under virus assays. Parallel control samples were incubated with pre-immunization serum in corresponding dilutions. The virus-serum mixtures tested in the absence of complement contained inactivated guinea pig serum (i.e., heated at 56°C for 30 min) in corresponding dilutions and volumes and these were also tested in parallel with the samples containing active (unheated) complement.

All sera were heated at 56°C for 30 min before use.

The neutralization rate constant, k, was then calculated from

$$k = \frac{2.303}{ct} \log \frac{v_0}{v}$$

where c is the final concentration of antiserum, t is the time in min of neutralization, v_0 is the initial virus titre and v the residual virus infectivity.

In some Figures, the $\log_{10}$ of the surviving virus fraction is shown plotted against time; in other cases, the residual percentage infectivity (% surviving virus) is shown i.e., the residual infectivity of the virus-antiserum mixture was expressed as a percentage of the infectivity of a mixture with pre-immunization serum at a corresponding dilution and after incubation for the same period.

Anti-herpesvirus sera

a) Rabbit immune sera

These were produced as described by Watson *et al.* (1966). Briefly, virus was grown in RK13 (rabbit kidney) cells which were then harvested, disrupted with an ultrasonic probe and the suspension finally freeze-dried. The immunizing antigen consisted of 0.2 g of freeze-dried material (equivalent to approximately 6 × 10^9 p.f.u.) mixed with 1.5 ml of incomplete Freund's adjuvant, the whole made up to 5 ml with sterile water. The resulting emulsion was homogenized and injected intramuscularly. To begin with, seven fortnightly injections were given, the first containing formalinized antigen. Bleedings were made 10 days after each injection and sera separated after standing the blood samples overnight at 4°C. (Sera are given laboratory identification numbers; thus serum 466/I is the sample taken 10 days after the first injection, 466 representing the rabbit number. Serum 466/III is the sample taken from the same rabbit 10 days after the third injection and so on). After the seventh injection, the rabbit was rested for three months and then boosted with 0.1 g antigen. It was then bled after 10 days, this sample representing serum VIII.

For the purpose of discussion in this study, the serum sample obtained from the first bleeding will be referred to as early rabbit serum. Sera taken from the seventh bleeding onwards will be termed late rabbit sera.

b) Human immune sera

Normal pooled human serum and paired sera from a patient (E.P.) were investigated for neutralizing antibodies against herpes virus in the presence of heated and unheated complement. In the latter case, the first blood sample was taken at the time of appearance of the herpetic lesions (early antiserum). The second blood sample was obtained seven weeks after the first and served as the late hyperimmune serum.

c) Mouse immune sera

Fifteen 3-month old mice were divided

Immune sera from rabbit No. 466

Serum No.	HSV type 1			HSV type 2				
	k_{+c}	k_{-c}	$k_{+c}-k_{-c}$	k_{+c}	k_{-c}	$k_{+c}-k_{-c}$	$k_{1+c}-k_{2+c}$	$k_{1-c}-k_{2-c}$
I	4.8	1.8	2.6	1.4	0.7	2.0	3.4	2.5
III	6.1	2.5	2.4	4.2	1.7	2.4	1.4	1.5
V	8.6	6.3	1.3	5.5	1.7	3.3	1.5	3.7
VII	8.3	3.8	2.1	7.2	3.9	1.8	1.1	1.0
VIII	24.1	20.5	1.1	6.8	3.5	1.9	3.5	5.7

Immune sera from rabbit No. 467

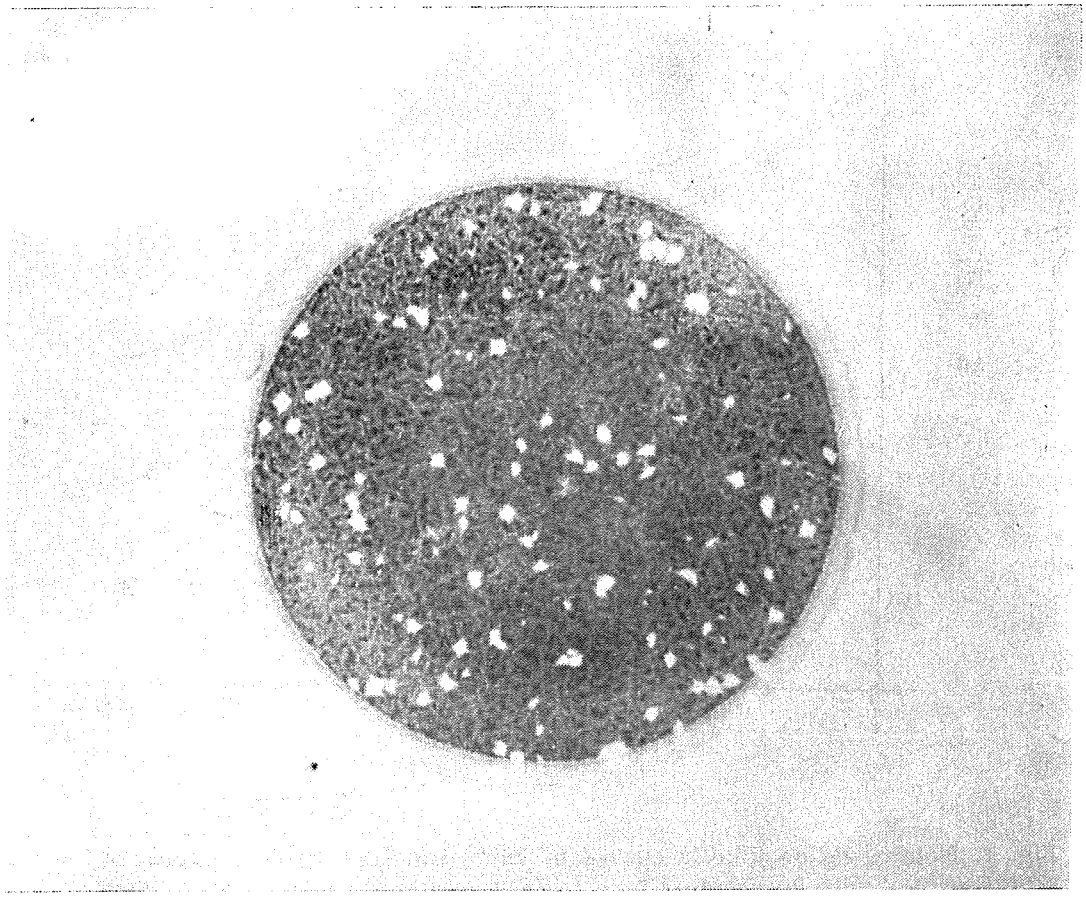
Serum No.	HSV type 1			HSV type 2				
	k_{+c}	k_{-c}	$k_{+c}-k_{-c}$	k_{+c}	k_{-c}	$k_{+c}-k_{-c}$	$k_{1+c}-k_{2+c}$	$k_{1-c}-k_{2-c}$
I	6.3	3.8	1.6	1.2	0.3	4.0	5.2	12.6
III	6.6	3.4	1.9	4.9	1.7	2.8	1.4	2.0
V	12.6	6.6	1.9	10.8	6.6	1.6	1.1	1.0
VII	14.7	6.9	2.1	10.8	6.4	1.7	1.3	1.1
VIII	16.6	10.1	1.6	6.6	2.8	2.3	2.5	3.4

Table 1. — The neutralization rate constant (k) values of immune sera from rabbits Nos. 466 and 467 with herpes simplex virus (HSV) types 1 and 2 in the presence ($+c$) and absence ($-c$) of complement.

into three groups and each immunized once with herpes virus type 1, each mouse receiving approximately 10^7 p.f.u. The virus was rendered inactive by treatment with 10% formol saline for 1 h at 37°C and injected intraperitoneally. All the mice in one group were killed and bled on the third day following immunization. The blood was pooled and the serum separated after allowing to stand for some hours at 4°C. The other groups of mice were similarly treated but bleeding was made on the tenth and twentieth day post-immunization.

Complement

Unheated guinea pig serum was used as complement. It was stored at -70°C until used. As indicated previously, heated guinea pig serum at 56°C for 30 min was added to those virus-serum samples which were to be tested in the absence of complement. Titration of complement was performed after the method of Yoshino and Taniguchi (1964). Unheated guinea pig serum was diluted serially in phosphate buffered saline. 0.05 ml of each dilution was then mixed with 0.05 ml of a virus suspension containing the same amount of p.f.u. as that used in the neutralization tests. A sensitized haemolytic system made up of equal parts of 3% washed sheep red cells and rabbit anti-sheep haemolysin containing 2 units was added to all tubes in 0.1 ml amounts. Appropriate controls were also included. After shaking, the tubes were placed in a waterbath



Plaques produced by Herpes simplex virus type 2 on BHK (baby hamster kidney) cells.

at 37°C for 30 min. Reading was made at the end of this period and the highest dilution of guinea pig serum giving complete haemolysis was taken to represent 1 haemolytic unit in 0.05 ml.

Results

Neutralization kinetic tests with rabbit sera

Five successive sera (I, III, V, VII and VIII), each from two rabbits (Nos. 466 and 467), were examined for neutralizing activity against both herpes simplex virus types 1 and 2 in the presence and absence of complement.

Table 1 shows all the data relating to these experiments. It will be seen that the k value of both rabbit sera against type 1 virus both in the presence and absence of complement was significantly greater than the corresponding value against type 2 virus. This fact can also be seen by examining the last two columns of the Table which relate to the ratio of the k value of type 1 to type 2 virus. This indicates that the sera were more specific to the homologous virus. The $\frac{k+c}{k-c}$ ratio (i.e., the ratio of the k value in the presence of complement to that in the absence of complement) of both sera, against both types of virus, showed no significant difference between the early and later sera. This indicates that no difference existed in complement dependence for neutralization between the early and late sera.

Fig. 1 shows the neutralization kinetic curves of early and late sera from rabbit

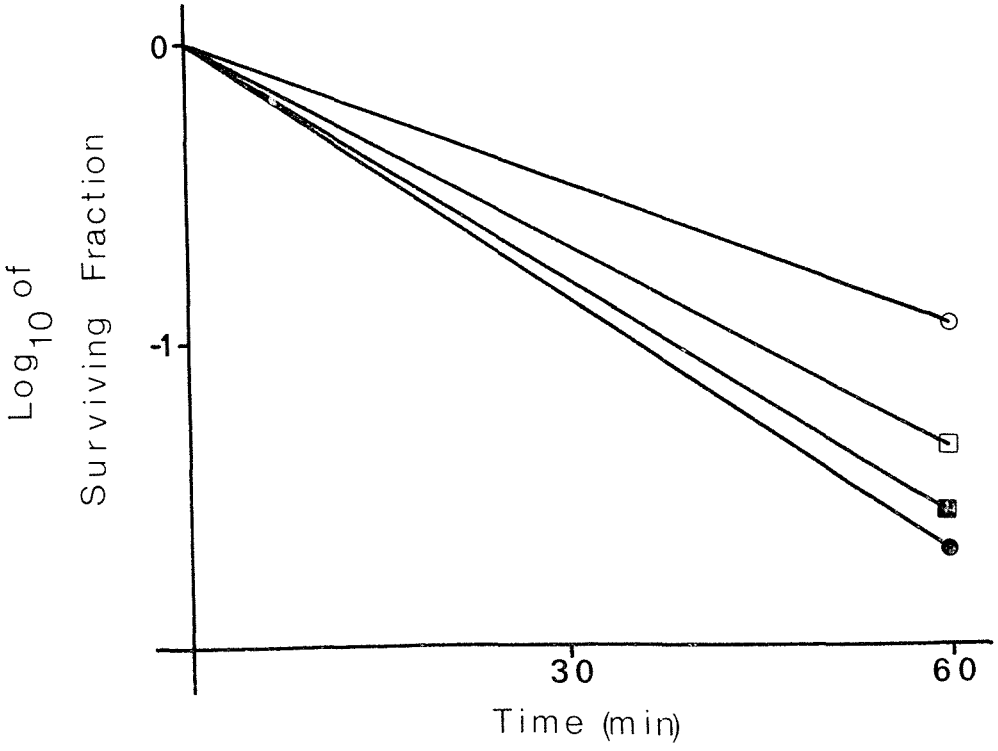


Fig. 1: Neutralization kinetic curves of early and late serum samples of rabbit no. 466 with herpesvirus type 1. Early serum (diluted 1/10): ○ with inactivated C′ and ● with unheated C′. Late serum (diluted 1/100): □ with inactivated C′ and ■ with unheated C′.

No. 466. Both sera showed a marked neutralizing capacity in the absence of complement which was only slightly enhanced when complement was added. The same effect was also seen when the same sera were tested against type 2 virus and also in tests with early and late sera from rabbit No. 467. Another, perhaps important finding, was that both early rabbit sera showed a relatively high k value when tested in the absence of complement (the average value against type 1 virus was 2.8 and against type 2 virus was 0.5).

The early rabbit sera therefore contained mainly non-CRN antibodies. The late rabbit sera obviously contained similarly non-CRN antibodies since neutralization was marked in the absence of complement. In both cases, neutralization was only slightly enhanced when complement was added.

Neutralization kinetic tests with human sera

Since it is known that a significantly high percentage of normal adults possess anti-herpes antibodies (Burnet and Lush, 1939; Yoshino *et al.*, 1962), pooled human serum was tested for neutralizing activity against herpes virus type 1 both in the presence and in the absence of complement. The result is shown in Fig. 2 It will be seen that the serum had relatively marked neutralizing activity even in the absence of complement.

Since pooled human serum is expected to contain a random mixture of early and late antiherpes antibodies, it was decided to test paired sera from patients known to have had a herpetic infection with a view to find out, if any, differences for complement requirement in neutralization between acute (early) and convalescent (late) sera.

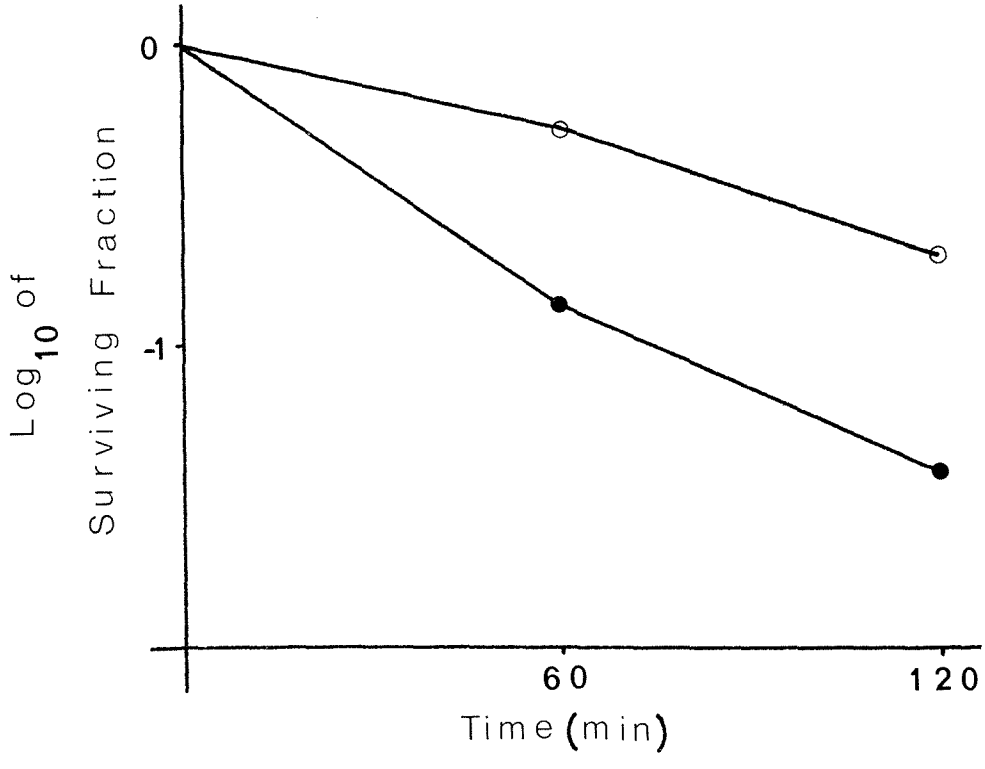


Fig. 2: Neutralization kinetic curves of pooled human serum diluted 1/15: ○ with inactivated C′ and ● with unheated C′.

Human immune sera

Neutralization data with HSV type 2

Serum (type and dilution)	k +c	mean	k −c	mean	k+c / k−c	mean
E 5/5	0.21		0.04		5.25	
E 3/5	0.27	0.34	0.02	0.036	13.50	9.50
E 1/5	0.55		0.05		11.00	
L 5/5	0.56		0.24		2.30	
L 3/5	0.80	0.94	0.35	0.420	2.30	2.24
L 1/5	1.47		0.67		2.20	

Neutralization data with HSV type 1

Serum (type and dilution)	k +c	mean	k −c	mean	k+c / k−c	mean
E 5/5	0.11		0.012		9.10	
E 3/5	0.12	0.17	0.012	0.018	10.00	9.30
E 1/5	0.29		0.030		9.00	
L 5/5	0.45		0.333		1.40	
L 3/5	0.60	0.67	0.311	0.390	1.90	1.70
L 1/5	0.98		0.533		1.80	

Table 2. — The neutralization rate constant (k) values of human early (E) and late (L) sera from patient E.P. with herpesvirus types 1 and 2 in the presence (+c) and absence (−c) of complement.

$$\text{For early serum: } \frac{k_{2+c}}{k_{1+c}} = 2.0 \text{ and } \frac{k_{2-c}}{k_{1-c}} = 2.0$$

$$\text{For late serum: } \frac{k_{2+c}}{k_{1+c}} = 1.4 \text{ and } \frac{k_{2-c}}{k_{1-c}} = 1.2$$

Paired sera from a patient (E.P.) were tested. This patient had both genital and oral herpetic lesions and a type 2 virus was isolated. The results of tests for neutralizing antibodies against both virus types and the effect of complement on the neutralization curves are shown in Figs. 3 and 4 and in Table 2. The k values were higher for type 2 than for type 1 virus both in the presence and absence of complement. This finding confirmed that infection was due to a type 2 herpesvirus. The early serum showed only a slight decrease in virus infectivity in the absence of complement. Neutralization was greatly enhanced on the addition of complement. In contrast, the late serum showed a marked neutralizing capacity in the absence of complement which was only slightly enhanced when complement was present. The mean value of the $\frac{k+c}{k-c}$ ratio for the early serum was more than 9.0 while that of the late serum was only approximately 2.0.

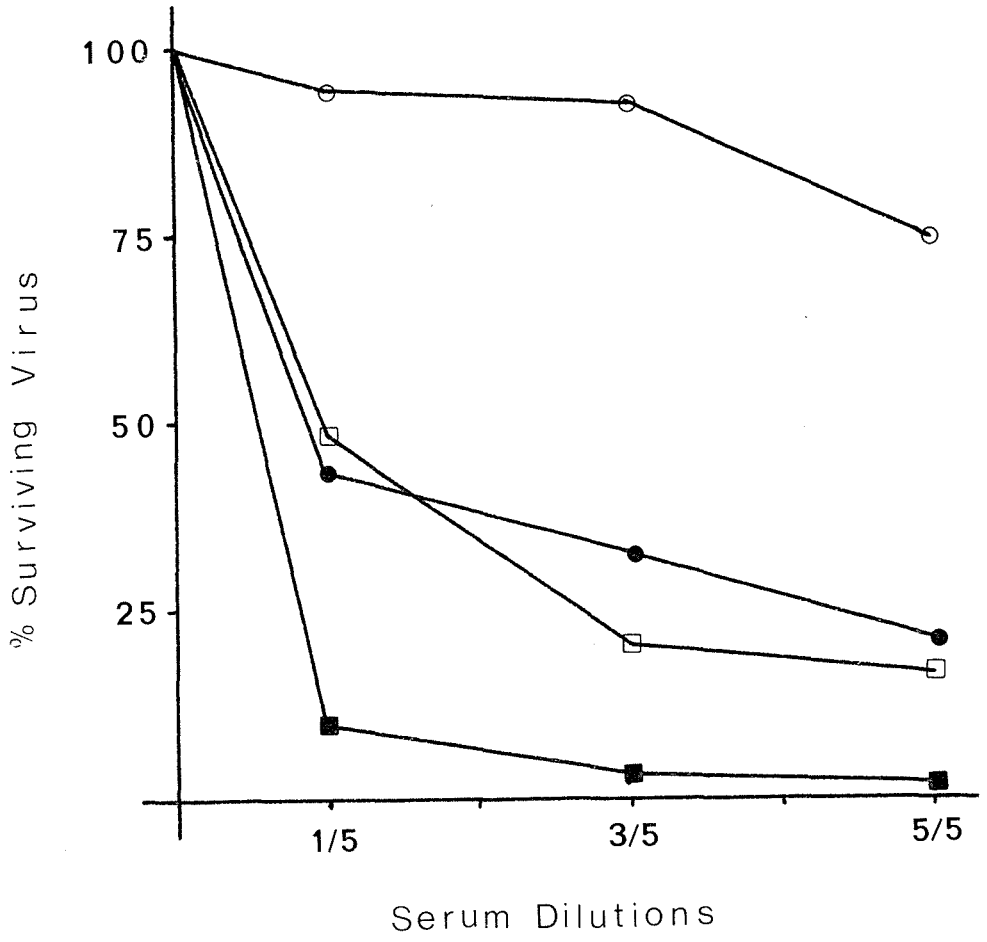


Fig. 3: Neutralization kinetic curves with Herpesvirus type 2 against serum from a patient (E.P.) with a herpetic infection. Surviving virus assayed after 30 min. Early (acute) serum: ○ with inactivated C′ and ● with unheated C′. Late (convalescent) serum: □ with inactivated C′ and ■ with unheated C′.

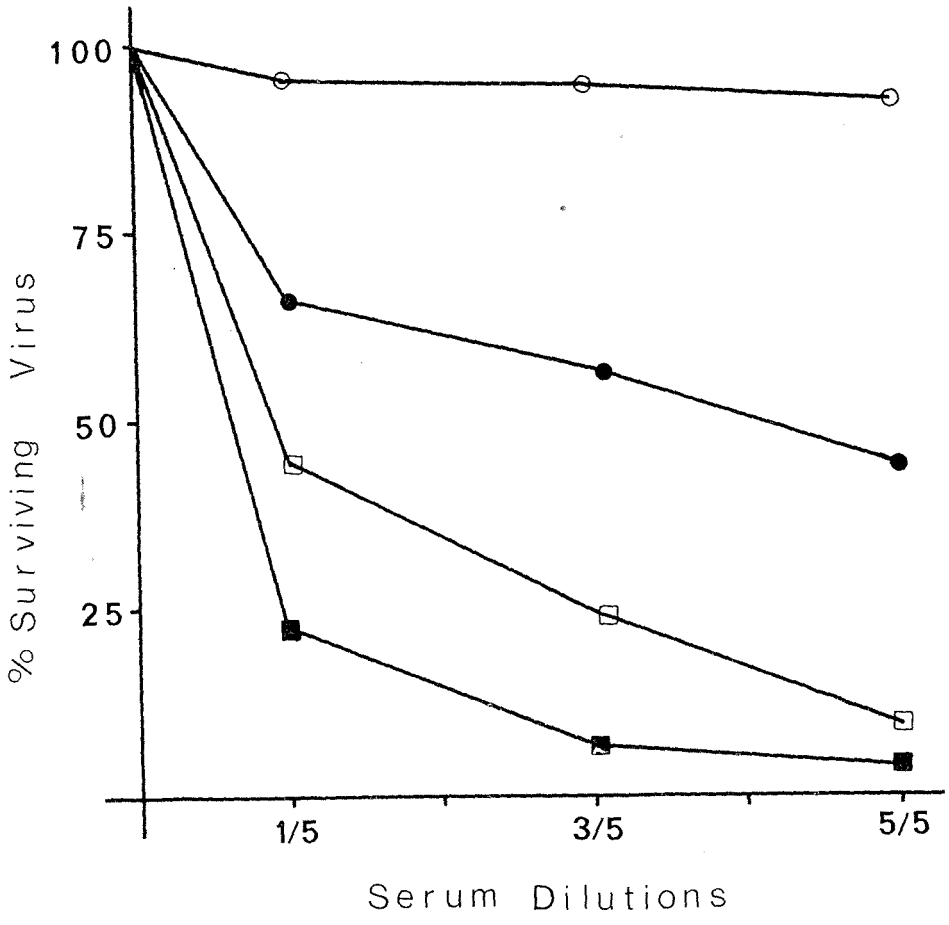


Fig. 4: Neutralization kinetic curves with Herpesvirus type 1 against serum from a patient (E.P.) with a herpetic infection. Surviving virus assayed after 30 min. Early (acute) serum: ○ with inactivated C′ and ● with unheated C′. Late (convalescent) serum: □ with inactivated C′ and ■ with unheated C′.

Neutralization kinetic tests with mouse sera

The results with early rabbit and human antiherpes sera differed in that only the latter contained CRN antibodies. The finding that the early rabbit sera did not contain CRN antibodies in contrast to results obtained by several workers (Yoshino and Taniguchi, 1964; Hampar *et al.*, 1968; Wallis and Melnick, 1971) may be due to the different method employed in this study in preparing the immunizing antigen, the route of administration and the time the sample was taken after the immunizing dose.

In an attempt to investigate this point further and to time the appearance of CRN antibodies, if any, mice were immunized with one dose of herpes virus antigen and successive blood samples collected as described earlier. The sera were kept at -20°C until all samples were taken and then tested simultaneously for neutralizing activity in the presence and absence of complement. The results are shown in Fig. 5 and in Table 3. Compared to the early human serum at the same dilution

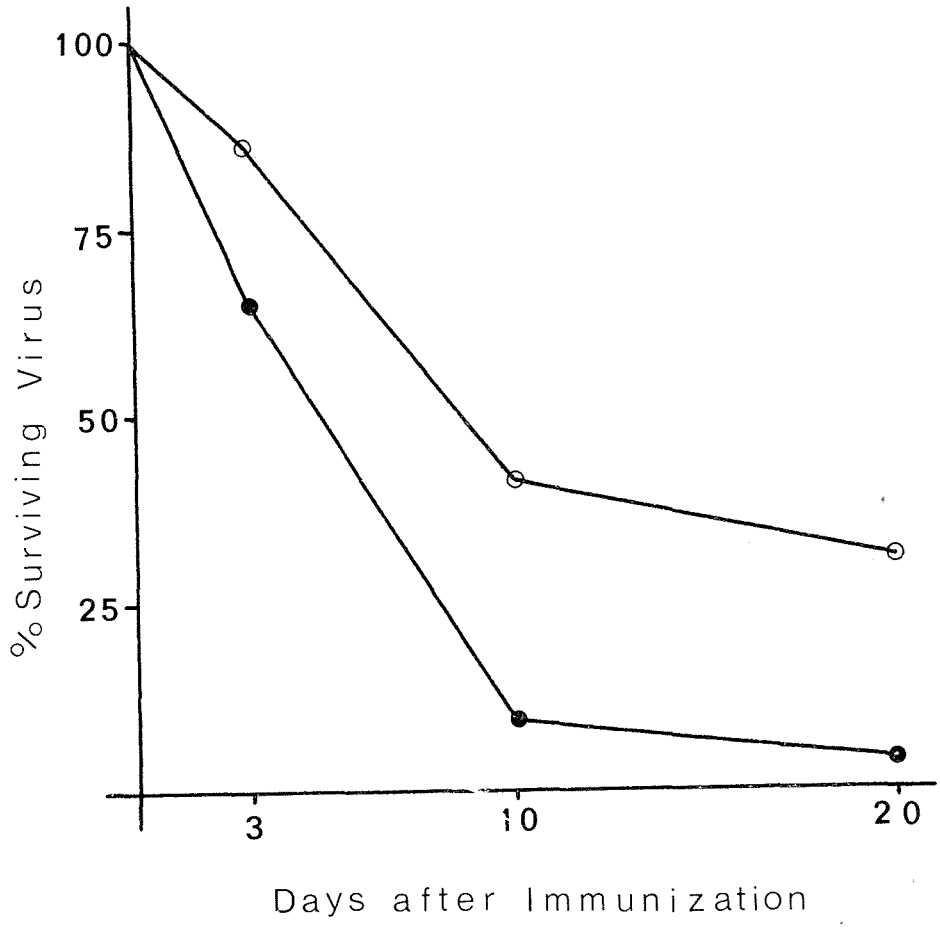


Fig. 5: Neutralization kinetic curves with mouse sera taken 3, 10 and 20 days after immunization with one dose of herpes simplex virus. In each case immune serum was diluted 1/5 and surviving virus assayed after 30 min.

○—○ neutralization in the presence of heated (inactivated) C′.

●—● neutralization in the presence of unheated (active) C′.

(Fig. 3), the first mouse serum sample taken on the third day after immunization showed moderate neutralizing activity in the absence of complement which was only slightly enhanced when complement was present. The later serum samples showed a marked neutralizing capacity in the absence of complement and enhancement occurred when complement was added.

The effect of varying concentrations of complement on the neutralizing activity of early rabbit and human sera.

Fig. 6 shows the results obtained in this experiment. Complement was diluted to contain the stated number of units in 0.05 ml. In other respects, the neutralization tests were carried out as described earlier. The early human serum showed little, if any, neutralizing activity in the

absence of complement. Neutralization proceeded on the addition of complement reaching a maximum at the 2.5 unit level after which excess of complement seemed to have no increased enhancing effect. In the case of early rabbit serum, neutralization was marked in the absence of complement and viral infectivity was reduced to approximately 20%. Neutralization was slightly enhanced by complement, again reaching a maximum in the 2.5-5 unit range and then remained constant even in the presence of excess complement.

Discussion

The experiments described in this thesis show that the neutralization of herpes simplex virus is enhanced by the presence of complement.

In most studies cited in the literature, dilution end-point neutralization tests were carried out to differentiate sera containing CRN antibodies from those containing non-CRN ones. In the present study, only kinetic neutralization tests were performed and this, perhaps, makes it more difficult to differentiate these two types of antibodies, especially in 'border-line' cases.

The early rabbit antibodies cannot be classed as complement-requiring for neutralization of herpes virus since neutralization was marked in the absence of complement. These results differ from those obtained by Yoshino and Taniguchi (1964; 1965a), Hampar *et al.* (1968) and Wallis and Melnick (1971) who showed that early rabbit antiherpes sera contained only CRN antibodies. This observation may reflect differences in the immunization procedures employed. Thus, the immune sera used in neutralization experiments by the above workers were raised by injecting virus intravenously without the addition of adjuvant and the early serum sample collected 7 to 8 days after the immunizing dose. The immune rabbit sera employed in this study were raised by injecting freeze-dried antigen in Freund's adjuvant intramuscularly and the early serum sample collected 10 days after the first immunizing dose. The method employed in preparing the immunizing antigen, the route of administration and the time of collection of the serum samples may thus be determining factors in the type of antibody which the particular serum may be expected to contain.

Mouse immune sera

Serum	k +c	k −c	k+c / k−c
3 day	0.29	0.12	2.4
10 day	1.69	0.57	2.9
20 day	2.10	0.78	2.6

Table 3. — The neutralization rate constant (k) values of mouse sera with herpesvirus type 1 in the presence (+c) and absence (−c) of complement.

Mice were immunized once with herpes virus type 1 and serum samples collected on the 3rd, 10th and 20th day after immunization.

All sera used in this experiment were diluted initially 1/5 and surviving virus assayed after 30 min of neutralization.

In the case of human infections with herpes simplex virus, the results obtained in this study suggest that antibodies present early in infection are dependent on complement for neutralization (CRN antibodies) while late sera contain non-CRN antibodies. These results were similar to those obtained by Yoshino and Taniguchi (1966) who proposed that detection of CRN antibodies in a single sample may become a new method for the early diagnosis of herpetic infections.

The mouse immune sera were raised by injecting antigen intraperitoneally and the earliest serum collected 3 days after immunization. The early mouse antibodies, in the absence of complement, were intermediate in neutralizing potency when compared, under the same conditions, with early human and rabbit antibodies. Enhancement of neutralization by complement was greater with early human than with early mouse serum. Later

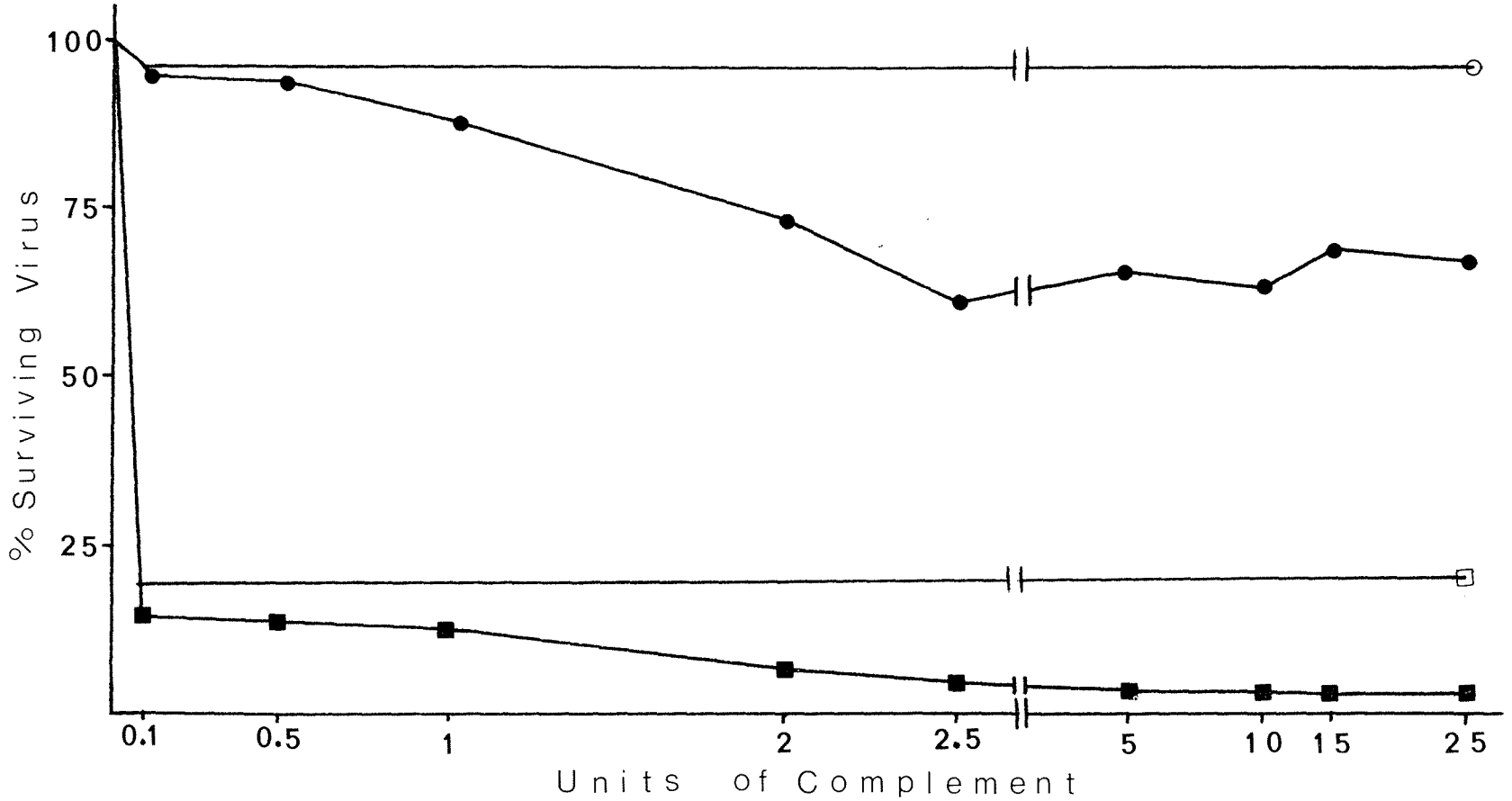


Fig. 6: The neutralization kinetic curves of early rabbit and human immune sera in the presence of varying concentrations of complement. Both sera were diluted 1/5 and surviving virus assayed after 30 min. Early human serum: ○ with heated C′ and ● unheated C′. Early rabbit serum: □ with heated C′ and ■ unheated C′.

mouse sera, taken from the 10th day post-immunization onwards, were shown to be progressively less complement requiring for neutralization. Yoshino and Taniguchi (1964) detected only CRN antibodies as early as the 3rd day after the immunization of rabbits. The statement that the first mouse serum sample similarly contained only CRN antibodies cannot be made with certainty. In this connection, it is perhaps worthwhile to mention that the virus employed in the immunization of mice was grown in BHK cells. The effect of BHK antigens on the immunization process or the presence of anti-BHK antibodies in the immune sera may have affected the neutralization of herpes virus by the mouse immune sera.

The question of the role of complement in the neutralization of viruses has been raised by many authors in the literature. Toussaint and Muschel (1962) showed that bacteriophage and antibody combined in the absence of complement but neutralization occurred on the addition of complement. Yoshino and Taniguchi (1965b) postulated two steps in the neutralization of herpes virus, the first step being irreversible binding between virus and antibody (which may still be infectious) and the second step, inactivation of virus. In the case of early immune serum containing only, or mainly CRN antibodies, the second step may be brought about by complement. The action of complement in this respect may then be similar to other antigen-antibody reactions involving complement such as cell lysis by antiserum (Oda and Puck, 1961). Berry and Almeida (1968) showed that avian infectious bronchitis virus, in the presence of antibody and complement, developed pits in the envelope which were very similar to those present on the membranes of sensitized erythrocytes lysed by complement. This suggests that the action of complement was to cause virolysis. Wallis and Melnick (1971) have proposed that the role of complement in virus neutralization was to cause immunoaggregates.

Hamper *et al.* (1968; 1971) showed that 7S and 19S rabbit antibodies to herpes simplex virus from early and late sera differed in their requirement for complement for subsequent neutralization. The early 7S and 19S antibodies showed low neutralizing activity in the absence of complement and greatly enhanced neutralizing capacity on the addition of complement. In contrast, both late 7S and 19S antibodies neutralized virus to a marked degree in the absence of complement although again, neutralization was enhanced when complement was added.

The early rabbit sera used in this study seemed to contain mainly late 7S and 19S antibodies since neutralization was marked in the absence of complement.

The early human antiherpes sera appeared to contain mainly early 7S and 19S antibodies as defined by Hampar *et al.* (1968) since neutralization was low or negligible in the absence of complement and greatly enhanced when complement was added. These antibody types were later replaced by late 7S and 19S antibodies as the late antiserum showed a marked neutralizing potency in the absence of complement.

The experiments with the mouse immune sera showed the sequential appearance of antibodies having successively less dependence on complement for the neutralization of herpes simplex virus as immunization progressed.

Acknowledgements

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SOME MORAL PROBLEMS ASSOCIATED WITH HUMAN TRANSPLANTS

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This paper is an abridged version of the St. Luke's Day lecture delivered to the Malta Branch of the British Medical Association on the 18th October 1972 at the Medical School of the University.

When dealing with the moral aspects of transplants one has to think of a double operation, that by which the tissue or organ is taken from one person, and that by which it is grafted on to another: hence we should consider both the receiver and the donor.

As regards the receiver the doctor should be sure that the patient will die if the transplant is not made, and that, besides, there is a good probability of the operation being successful. The surgeon should also have the consent of the patient, or, if he is a minor, of his parent (father) or tutor.

As regards the donor, the transplant may be an autograft, or a graft from another person. In the first case there is no difficulty as all the parts of a living human organism are ordained to the benefit of the whole. The surgeon has only to be morally sure, medically, that both excision and graft are necessary for the patient's survival and scientifically likely to be successful (cf. Fr. Gerald Kelly, "Notes on Moral Theology, 1951", in *Theological*

Studies, March, 1952, p. 60; Pius XII's Address to the First International Congress of Histopathology of the Nervous System, September 13, 1952, in *Addresses of Pope Pius XII*, 1952, Edizione Paolini).

The problems arise with grafts from another individual. The donor may be dead or alive. In the former case the consent has to be given by the next of kin or the situation must be such that the consent may be legitimately presumed. Obviously the case in which it cannot be presumed would be when the dead donor had expressed such a refusal in his will. Still, even in such a case, if the necessity of saving the receiver make the measure urgent and no other provision for a transplant is possible, one might perhaps agree to presume that the dead donor would not have been unwilling to change his mind had he been still alive.

The crucial problem is how to be certain that the donor is dead, because if he is not, even though his state is hopeless and it is certain that he would die very soon, an excision made upon him which terminates his life is murder. The traditional definition of death used to be the complete and permanent cessation of respiration and circulation, but recent advances in chemical and electronic cardiac resuscitation techniques as well as the use of mechanical respirators have created a need for a new definition. In the case of death through an accident, the determining of death may not be difficult, for if, in that case, the corpse shows damages or lesions which are incompatible with life, e.g. if the brain is completely crushed, or the head cut off, it is clear that death in its absolute sense, is present. In fact, the soul can only animate a functional body, i.e. one which has the essential organs for animation, for life; and that requires that the body still retain those of its organs which are required for animation. Hence if it lacks the brain or the heart it is not in a state to be animated by a soul. If these essential parts are lacking, even though there is still some movement, artificial or otherwise, in other parts, the body as such is dead and the soul will not animate it.

Clinical death is now generally accepted as identifiable with brain-death, and the electrocephalograph is the standard instrument to detect it. But even here one may safely ask: Is this absolutely safe? Indeed, a barbiturate dose may result in a flat EEG for four hours or more, and nobody will call that death. We might perhaps define death as the point at which the brain ceases to function having reached a point of no return, i.e. having reached a stage when it is unable to regain its functionality. The electroencephalograph is certainly a very helpful instrument for establishing that state provided that the straight EEG line is not provoked by artificial agencies, such as the overdose referred to above. Decomposition of the body begun and permanently continued certainly indicates death, but it is more an indication of a state of death, not of the moment at which death occurs; and for transplant operations the state of decomposition may be too late: the surgeon wants the organ to be transplanted *before* decomposition begins.

The case of transplants from human beings who are still alive raises greater problems. If what is taken from the donor destroys or substantially impairs the biological functionality of the organism, provoking death, then or later, the excision is not morally allowed, because it amounts to murder if the donor is not willing, and to suicide if he is willing, since man has only the use, not the ownership of his own body. This has always been the teaching of the Catholic Church, and Pius XII confirmed it in the address quoted. These are his words: "As regards the patient, he is not the absolute owner of his own body and of his soul. He may not therefore freely dispose of himself as he pleases or fancies... the patient is subject to the Theology which follows from nature; he has the right to make use of his body, limited by the natural scope of the faculty itself... and because he has the right of use, he is not the owner, he has not an unlimited right to accomplish acts of destruction or of mutilation of an anatomical or a functional character."

If what is taken from the donor does not destroy or substantially impair the biological and functional integrity of the organism one should distinguish. If what is

taken is minimal and clearly does not involve mutilation in the strict sense of the word, and, besides, does not impair one's health, e.g. a small bone, a small piece of skin, a blood transfusion or the like, there is no moral prohibition whatever. But theologians are divided in their opinion of the moral correctness of the act if what is excised is a whole organ (one, which, if single, does not provoke death if excised, e.g. the uterus; or both of a pair if they do not provoke death when excised, e.g. both ovaries; or one of a pair, e.g. a cornea, a kidney, etc.).

Some theologians consider such an excision as *always* immoral since, in their view, it runs counter to God's ownership of the human body. They think that every mutilation is a violation of biological integrity, and they appeal to the maxim *"non sunt facienda mala ut eveniant bona"* (what is evil may not be done for the sake of a good purpose). Furthermore they refer to the statement of Pope Pius XII quoted above and to the encyclical *Casti Connubii,* paragraph 24 (30th Dec. 1930) of Pope Pius XI which reads as follows: "Christian doctrine establishes, and the light of human reason makes it most clear, that private individuals have no power over the members of their bodies beyond that which pertains to their natural ends; and they are not free to destroy or mutilate their members, or in any other way render themselves unfit for their natural functions except when no other provision can be made for the good of the whole body." Theologians quoted as holding this view are L. Bender, *Organorum humanorum transplantatio,* in *Angelicum,* XXXI (1954); *Il trapianto di un membro dal punto di vista morale,* in *Perfice Munus,* (Aprile, 1955); T. Goffi, *Moralità del trapianto di un membro umano,* in *Rivista del Clero Italiano* (1954); *idem* (1955); G. Borg, *Del trapianto di un membro,* in *Perfice Munus* (Marzo, 1955); F. Regatillo-M. Zalba, *Theolagiae Moralis Summa* (1953), Vol. II, n. 252; F. Hurth, *De re matrimoniali,* (1955).

Another group of theologians, while accepting that mutilation is illicit, are of the opinion that there can be exceptions "when God is expected to be willing", and they hold that God may be considered to to be willing when the excision is done for the noble motive of charity, taking, of course, account of due proportions. The unity of the human race in the brotherhood of creation and of Redemption would be the justification of the charity motive. Prominent theologians holding this view are A. Vermeersch, *Theologiae Moralis Principia,* 1945, Vol. II, N. 299; P. Bongiovanni *Trapianto di un organo,* in *Perfice Munus* (dic. 1954); A. Gemelli, *Sulla liceità di cedere un organo per il trapianto omoplastico,* in *La Scuola Cattolica,* 1934; D. Gennaro, *Casus conscientiae,* in *Perfice Munus* (Aprile, 1955).

Still another group of theologians who accept that mutilation, if considered in itself, is immoral, but who allow transplants of one of a pair of organs, redefine mutilation. They hold that by mutilation should be meant not the excision of an organ, but the destruction of its functionality. In their view, if an organ was excised in such a way that it remained alive and functioning, and hence, when grafted, continued to function, biologically, as newly grafted, one could not speak of mutilation. Apart from charity motives as in the precedent theory, they quote in their favour the *Casti Connubii* passage of Pius XI already mentioned. They contend that from the Pope's words one can gather that bodily organs are given to man for their natural end, i.e. for a definite biological purpose, and the words "or in any *other* way" equate the word "mutilation" with "unfitness for their (the organs') natural functions". Hence, these theologians think it is immaterial on which body the organs function as long as they continue to function properly (given always a good reason — the motive of charity — and taking care of due proportions). Holders of this view are L. Babbini, *Il trapianto delle ghiandole alla luce della morale,* in *Palestra del Clero,* 1950; L. Scremin, *Dizionario di morale professionale per i medici,* 1953; and the already quoted Bongiovanni and Gennaro.

Lastly, another group of theologians, and quite a large one, holds that mutilation is in itself not bad and illicit, but

simply morally indifferent. They contend that if man may offer his *life* for the good, spiritual or material (duly proportionate) of others, why not also an organ of his body if this does not provoke death? They quote Our Lord's words (John, 15, 13): "Greater love than this no man hath, that a man lay down his life for his friends." The Church has canonised Fr. Kolbe for giving his life to save a family man (from motives of divine and human charity, of course, which Fr. Cunningham expresses in terms of the unity of men in the mystical body of Christ). The execution of condemned murderers by public authority can only be morally allowed because it is presumed that "God is willing" and because the taking of life is not of its own nature *(ex natura sua)* bad, otherwise God would not be able to allow it. Some moralists ,among them St. Alphonsus de Liguori (whom the Church has made the patron saint of Moral Theologians), have not deemed it possible, morally, to condemn castration carried out with the object of keeping a childlike voice. Even granting that man is not the owner of his body, yet he is its good administrator. This allows him to beat his body even to the shedding of blood and to deny it nourishing food for the spiritual purpose of self-chastisement and self-denial, and it also allows him to accept some mutilation and to dispose of some organs for the spiritual and material benefits of himself and of his fellow men. The biological integrity of one's body is not substantially impaired since the excision of one of a pair of organs does not involve death or ill-health, and the excised organ fulfills what might be called a secondary natural function in its new body without defeating its primary purpose which was the good of one's own body.

Papal pronouncements should be interpreted within the framework and context of the purpose and occasion of their delivery. In *Casti Connubii* Pius XI was referring to the sterilisation laws proposed by Hitler and had no intention of referring to transplants, apart from other considerations of interpretation as above. Pius XII was referring to the same methods by which a cure was being sought to maladies of the nervous system (of a mental nature) by the suppression, even temporarily, of personal liberty or by the excitement of sexual instincts. Pius XII does not say that man has no power whatever of mutilation, but, on the contrary, he says that man has *not* an *unlimited* power of mutilation. Hence, it follows, that, albeit limited, he does have some power of mutilation in terms of the anatomical and functional characteristics of his organs. All of it makes it clear that the Pope was speaking of *substantial,* not absolute integrity of the body's biological functioning. As a result of these considerations these theologians formulate their argument in the "double effect" form: from mutilation which is morally indifferent, two effects follow independently, the positive one which is to save life, and the negative one, the justifiable loss of a member of the body.

This last theory has been upheld by many theologians right to the present day. We mention B. J. Cunningham, C.M. (The Morality of Organic Transplantation, Washington, 1944); G. Kelly S.J. (Notes on Moral Theology, 1946; The Morality of Organ Transplantation, in Theological Studies, VIII, 1947, and in XI, 1948); "Preserving Life" in *Linacre Quarterly,* Feb. 1957); McCarthy, (The Morality of Organic Transplantation, in *Irish Ecclesiastical Record,* Vol. 67, 1946); J. Connery, (The Morality of Organ Transplantation, in *Theological Studies,* XV, 1954); J. Pereda (La Mutilacion y el transplante de organos, in *Estudios de Deuste,* julio-diciembre, 1954); M. V. Murray, (Problems in Conduct, N. York, 1963); J. J. Lynch, (Ethical Implications of Renal Transplants, in J. Amer. Med. Assn., April, 1967; Ethics of the Heart Transplant, in *America,* Feb. 1968); C. Curran, (The Morality of Human Transplants, in *The Sign,* March 1968); B. A. Paparella, (Moral Principles Applicable to Organ Transplantation, in *Linacre Quarterly,* Nov. 1968). Since the Church has not condemned these views (of this last and fourth group of theologians) expressed after, and in interpretation of Pope Pius XII's statement, and as the arguments advanced command attention, their opinion

is probable enough to be safely followed, from the moral point of view, at any rate until the position is definitely clarified. Thus Fr. Lynch (op. cit.) writes: "No one is required to acknowledge as obligatory a prohibition which is at best objectively doubtful. In other words, no theologian could legitimately accuse of moral wrong doing the physician who involves himself professionally in organic transplantation with due regard for those precautions which sound medical sense would prescribe for that procedure. Or to put it more precisely... the doctor, who in his prudence seeks to preserve human life by means of organic transplantation, can merit no less theologically than he does scientifically, and Fr. Curran (op. cit.) says: "The general principle would be that one has administrative power over one's own body, and that, provided he does not appreciably maim himself or lay himself open to the risk of personal injury, he can give what he has to help someone else."

SOME MALTESE PHARMACISTS OF THE PAST

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In England pharmacy has been for centuries practised as an art by itself. In 1180 a "Guild of Pepperers or Spicers" already existed. In 1345 pharmacists formed a separate section of the Grocers' Company and in 1671 the "Worshipful Society of Apothecaries" was founded and received a charter from King James I. A Royal Apothecary probably existed since the days of William the Conqueror and certainly officially since the reign of King John, his duties consisting in compounding medicines for the Royal household, providing the palaces with the necessary wine, spices, drugs and perfumes, and eventually embalming his employers.

In Malta until late in the period of the Order of St. John pharmacy was practised as part and parcel of the art of medicine and often by the knights themselves in their large and celebrated hospital. The name of Pharmacist Chevalier Anthony de Lande appears in the records as one who, together with officials of Monsignor Dominic Petrucci (the Inquisitor-General who was in Malta from 1579 to 1581) and some knights had been maliciously reported to Grand Master de La Cassiere as having accepted a proposal to prepare a poisonous potion for him. The charge had been made by Impellizeri, a Sicilian who had been banished from Malta by the Inquisitor. La Cassiere ordered the imprisonment of the plotters in Fort St. Angelo from where they were freed when it was discovered that the Sicilian had fabricated the whole story in the hope that his previous sentence of perpetual banishment would be abrogated.

Here are some pharmacists of the past in Malta, such as could with difficulty be discovered.

Stanislas Gatt (1744-1811) had a pharmacy at Qormi near the small church of St. Peter's in the centre of the small town. He obtained the diploma of "Aromatario" from the Chief Physician or Protomedico of the Sacred Infirmary of the Order on the 13th April 1771 (Arch No. 1192, Section 86/97) during the grand-

mastership of Pinto who had raised Qormi to the rank of a city and given it his name. Gatt who had originally studied for the priesthood had changed over to pharmacy and in 1778-1780 was chosen as a "Jurat". He is mostly remembered as the leader of the 5th Municipality (Qormi-Luqa) and the "Sindaco" for Qormi, during the uprising against the French in 1798. The French had burgled the church of St. Francis de Paule breaking down its door, tearing out holy pictures and stealing the church silver. Gatt's daughter died on the 3rd March 1811 and, broken hearted at her loss, he died the very next day.

Clemente Mifsud Bonnici was one of the pharmacists at Zejtun, *"Aromatario e dilettante di medicina"*, who also had obtained his pharmaceutical diploma from the Protomedico Dr. George Imbert and from Dr. Michael-Angelo Grima. Clemente Mifsud Bonnici was the son of Joseph Mifsud and Mrs. Olympia neé Bonnici. In 1816 he married Theresa Falzon. He was the friend of Stanislaw Gatt and like him, during the despotic French rule, was selected by the inhabitants of his district (which had just lately, on the 30th December 1797, been elevated to the status of a town by the German Grand Master Fra Ferdinand von Hompesch (1797-1798) as "Citta Beland", so called after the Grand Master's mother maiden name "Bylandt") along with his comrade the Architect Michael Cachia (1760-1839) the son of the more famous Architect Dominic (1710-1790), as their co-leaders against the oppressors. Mifsud Bonnici was also one of the chief helpers of Santo Formosa from Zurrieq and took part with him in the assault against Mdina on the 13th September 1798. He likewise did sterling pharmaceutical and humanitarian work during the great plague epidemic of 1813-1814, when the dread of catching the "contagion" had been so great that priests used a metal spoon with a long handle to administer Holy Communion to the stricken patients so that they could remain at some little distance; indeed dying or dead victims were not touched by hands but were loaded on to the death-carriage and buried mostly by hooded convicts using wooden or iron implements such as rakes and tongs; it is recorded that 13 medical practitioners, including Dr. Saviour Pisani of Vittoriosa, Dr. Michael-Angel Pace of Gozo and George Craig MacAdam, an English fellow doctor, died of the disease.

Another pharmacist was Francis Pisani who, incidentally, was married to the reverend professor Michael Xerri's (the well remembered "Dun Mikiel Xerri") niece Theresa. He openly showed his mistrust of the French overlords to the Senglea population, whom he incited to disobey their strange orders; his sentiments were unfortunately reported to the French Authority by a francophile neighbour, a frequent visitor to his dispensary and he was mercilessly shot in August 1798 suffering the same fate which befell his uncle by marriage on the 17th January 1799.

Fortunato Vella had been given his diploma of "Aromatario" by the same Medical Council of the Order's General Hospital headed by Dr. George Imbert and composed of Senior Physicians Dr. John-Dominic Blasio, Dr. Joseph Bigeni and Senior Surgeon Dr. Michael-Angelo Grima, on the 23rd September 1770. Vella's diploma can still be seen hanging on the left-hand wall at the top of the main staircase of the Royal Malta Library.

The next oldest diploma after Vella's, one about 50 years later in date, is in the possession of Mr. Joseph Galea of Mdina. It is that of Paul Stivala, who was given his diploma on the 6th August, 1817, during the Governorship of Sir Thomas Maitland (1813-1824), when Dr. Aloysius Caruana was "Protomedico".

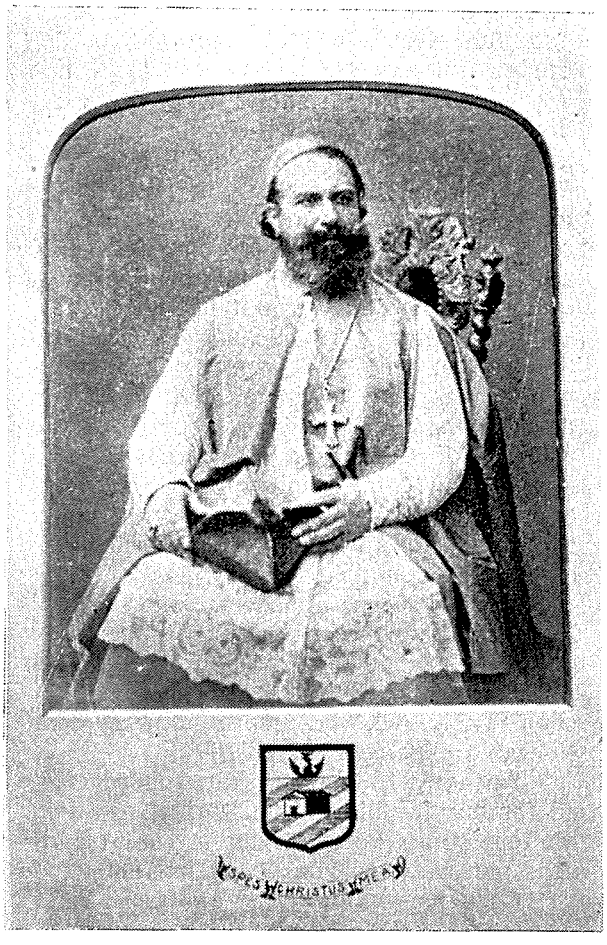
John Mallia, (1774-1856), from Birkirkara had his pharmacy in St. Helen's Square of that small town. He was a bachelor and rather wealthy, possessing a very large estate including some lands known as "Tad-Dekan" at Msida, colloquially referred to as "Tat-Tigan" ("a frying pan", a word more commonly used by the country people than the name of a dignitary of the Church chapter). It is said that Mallia had a very large clientele, since he charged less for his medicines than his colleagues did. It is a fact that he left several other houses and fields to the

church and to church charities. His pharmacy, complete with its fixtures, furniture, procelain jars, etc. he bequeathed to the Floriana Franciscan Capuchin Fathers on condition that a yearly liturgical service for the dead and for the repose of his soul, should be held, as appears in the Acts of Notary Peter Paul Psaila of the 11th June 1855.

It was quite understandable that his best friends were the Provost of Birkirkara Canon Calcedonius Agius from 1854 to 1867, Canon Michael Tonna his testamentary executor, Canon Constantine Sant, the Reverend John Micallef, the Reverend Horatius Galea and the Cleric Francis Sammut, besides Dr. Anthony Cassar, M.D., all of whom were enjoined to pray for his eternal rest.

Andrew Debono (1821-1871), from Senglea, the son of Captain Michael Debono and Theresa Carabott, studied in Malta and obtained his diploma from our University, but practised his profession at the hospital of Rosetta in Egypt. He married Victoria neé Birella and had two sons, Edward and Alfred. His great achievement was as an explorer because during 1845-1855 he voyaged along the Nile and was fortunate and strong-willed enough to arrive at its remotest source at the head of the Kagera river near Lake Tanganyika at 4037 miles from its Mediterranean end, a feat in which so many others had failed. For some years he lived at the main White Nile station. While passing through the Sudan along the cataract or rapid near Khartoum, Andrew Debono paid a visit to the Apostolic Vicar there, our co-national His Lordship Monsignor Annetto Casolani, D.D., Titular Bishop of Mauricastro (1815-1866) son of Sir Vincent Casolani, G.C.M.G. who incidentally had built the Bishops' Palace at Khartoum, later used by Major-General Charles George Gordon (1833-1885), who was killed in the Mahdi's rebellion there. On account of ill-health Bishop Casolani retired to Malta where he died on 1st August, 1866 and was buried in the Cathedral Church at Mdina.

Andrew Debono is mentioned in Alexander Maitland's book 'Speke' (Constable), where he is described as a "Maltese slave-trader who operated in the southern



Bishop Annetto Casolani

Sudan". On his sepulchral slab, at the old Cairo City cemetery, he is described as "a good and honest man". Debono's nephew, Mr. Amabile Debono, is also referred to as "a Maltese ivory trader who was the first European to have entered the bounds of Uganda".

Andrew Debono's son Mario, who was also a Pharmacist at Bone in Algeria, became a friend of the famous Gozitan-born French writer Laurent Ropa (1891-1967) and of the perhaps even more famous Frenchman of Maltese grandparents, the poet and Academician Fernand Grech (1873-1960).

Lawrence Dalli, from Gozo, obtained his diploma signed by the Protomedico the

Maltese Dr. Gabriel Henin who had specialized in Florence on the 26th January, 1832 (ch. Arch. No. 1187, Section 310/317) during Vilhena's grandmastership. I believe that this pharmacist who lived, according to the famous Gozitan historiographer de Soldanis, in the Renaissance house under the archway in the Citadel near the old shrine of Our Lady of Sorrows, which has since been converted into a Museum, was most probably the first qualified pharmacist in our Islands.

Augustine Xara had some reputation for having, in 1763, concocted a poultice which was said to have been useful in the treatment of "angina pectoris". In the manner of the times this poultice was made up of Maltese honey, hens' fat, flowers of wild thyme, tobacco ash and even the dried excreta of doves, to which some almond oil could be added.

Carmel Arpa, a pharmacist son of Francis-Xavier Arpa, who was a professor of Midwifery, in 1889 bought the Manoel Theatre, which he later sold to the Gollcher family, who re-decorated it at considerable expense in 1906-1907.

Anthony Emmanuel Caruana remained famous for his fine, historical novel in Maltese "Inez Farrug", set in the Aragonese period, which he wrote in 1889.

Caruana was born at Valletta on 22nd April 1838. He practised his profession at the pharmacy belonging to his maternal uncle, Dr. Andrew Pullicino, under the arcades in Valletta. He liked travelling and in 1860 went as far as Constantinople and even to Russia. He learned several languages, including Russian, and mixed with many men of letters both here and abroad. Caruana married Connie Fabri of Cospicua in 1883 but had no offspring. He died at Sliema on the 24th September, 1907.

Augustine Levanzin, (1872-1955) a man with a complex character, had qualified as a pharmacist. Levenzin first worked as an apprentice at the Dockyard, where he was promoted to clerk and then to draughtsman, but which he left to join the Seminary. After matriculating he joined the University Course of Medicine and Surgery. He obtained a B.A. degree and a diploma of Pharmacist. In 1900 he married the daughter of Dr. John Felix Inglott

Dr. Philip Zammit

of Cospicua. Then he rejoined the University obtaining this time the diploma of Legal Procurator. He was the editor of "Lo Studente Maltese", of "Il-Habib tal-Poplu" and of "In-Naħla" in which latter he published his novel "Is-Saħħar Falzon". A history of Joan of Arc he published in the excellent series "Mogħdija taż-Żmien" (No. 89). In 1912 he emigrated to the United States of America where for physiology studies he underwent a fast of 31 days. Back in Malta in 1927 Mr. Levanzin upheld that "Fasting promotes Health", but as he got involved in politics, he preferred to go abroad again in 1928. At one

time he was keen on "Esperanto" — the international language. He was a staunch defender of workers in general. He died aged 83 in 1955.

It is usually held that not before the first third of this century had passed was there a lady-pharmacist in our Islands; this, however, is not correct as the following exceptional case clearly illustrates. Catherine Vitale, "La Speziala", was the widow of Hector Vitale and lived at 138 Archbishop Street, Valletta. It is said that this lady, after the death of her husband, opened a small pharmaceutical store in Valletta for the use of poor patients, which earned her her appellation. She used mostly local drugs from which she then concocted medicinals in her kitchen. Her example was so successful that a little later in 1642 her friend, a spinster Catherine Scappi from Siena, Italy, (hence known as "La Senese"), of 144 Archbishop Street, Valletta, opened "La Falanga" a small institution for foundlings and the adjoining "La Casetta", a place for poor and chronically sick women, For this she came to be referred to as "La Spedaliera". On her death-bed in 1619 Catherine Vitale bequeathed all her property, including the site for the original building of Selmun Tower on the approach to Mellieha, to the famous *"Monte della Redenzione degli Schiavi"* or Bank for the Ransoming of Slaves carried off by the infidel, which she had started in 1607. She was buried at the church of Our Lady of Mount Carmel at Valletta, as was later her friend Catherine Scappi: the respective marble tablets on their graves were fixed in the wall of the passage leading from the church to the sacristy for better preservation when recently the church began to be rebuilt.

Balthassar Debono from Zebbug, although a qualified doctor preferred to practise as pharmacist as several others did after him. He is best remembered not for his pharmaceutical expertise but for his founding at Zebbug in Malta the church of "Our Lady of Sorrows" in 1720 for which church he obtained in 1726 the honour of being designated a basilica.

Philip Zammit (1719-1796) from Zurrieq, obtained his diploma in Pharmacy and later his doctorate in Medicine and Surgery at the Order's Xenodochium from the Protomedico and his assistants Doctors Grima, Locano, Grech and Henin. Dr. Zammit was appointed Medical Officer incharge of Santo Spirito and Saura Hospitals at Rabat. As a pharmacist he invented a health-giving salt and an antacid-stomach powder which were in great demand here and even in Sicily. For his chemical, pharmaceutical and medical capabilities he was highly praised by Baron Cajetan Pisani of Malta, Abbot Malherbe and the famous Father Mussio of Naples. When he died in 1796, he left his wealth to Saura Hospital which generous exam-

Sir Themistocles Zammit

ple was later followed by his daughter Catherine. Oil paintings of them at Saura Hospital testify to gratitude for their benefactions. Dr. Nicholas Saura from Siggiewi, who founded this Institute on the 17th September 1639 and who died in 1654, and his daughter Isabella, who also left her patrimony thereto, are similarly remembered.

To conclude this list with a famous name we point out that it is not generally known that Sir Themistocles Zammit (1864-1935) started his highly varied and successful working life as a pharmacist. He was first employed in a private pharmacy and later at the Central Civil Hospital in Floriana during the cholera epidemic of 1887. He had also attended a special course at the "Ecole Superieure de Pharmacie" in Paris.

THE FIRST SEVENTYFIVE YEARS OF RADIOLOGY IN MALTA

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On the 8th November 1895 William Conrad Röntgen (1845-1923), Professor of Physics at the University of Wurzburg, discovered X-rays. He presented a preliminary communication on the new type of rays to the Physical Medical Society of Wurzburg on the 28th December. On the 6th January 1896 the news of his discovery appeared in the lay press. By the 30th of the same month the importance of the new rays in the diagnosis of fractures and the location of foreign bodies was recognised (Bleich, 1960; Snyder, 1967).

News of the discovery of X-rays or of the "New Photography", as they were also called, appeared in Malta on the 12th March 1896. The editor of *The Daily Malta Chronicle* of that date expressed his regrets that he was not able to provide his readers "with full particulars of the recent advances made" but hoped to do so within a few days following the return of his Science Editor from a short vacation in Morocco. For unknown reasons this write-up never appeared. On the 13th March the same newspaper announced the conferment of the title of Doctor of Medicine on Röntgen by the University of Wurzburg in the following terms: "It is not often that a prophet or an inventor either, for that matter, is speedily honoured in his country; but Röntgen of rays renown has had a different expe-

rience happily for himself and those who have delighted to do him honour. Last week a deputation of a Medical Faculty of Wurzburg University, of which he is a professor, presented him with a diploma conferring the title of Doctor of Medicine, the degree having been granted unanimously."

Snippets of information about Röntgen's further experiments and their successful application to medical diagnosis filtered through to Malta at irregular intervals and appeared in the press during the year *(The Daily Malta Chronicle,* 1896).

By early November 1896, and probably earlier, experiments with the new rays were being carried out in Malta by Mr. John Ellis of the photographic firm Richard Ellis at 43 Strait Street, Valletta.

On the 5th November, Mr. Ellis wrote as follows to Count Gerald Strickland, Chief Secretary to the Government of Malta (1888-1902). (We reproduce verbatim):

"Sir,

I am sending you two more copies done by the X-rays of 'this week's experiments'.

The No. 1 copy is interesting as it shows the different nature and approximate size of thickness and height of the cases.

No. 1 aluminium cigarette case— gold chain and ring of which only the later *(sic)* shows.

No. 2 A leaf of which only the fibre shows — placed partly under Nos. 1 - 4 - 3 and direct to the light in other parts.

No. 3 Tortoise shell money case silver mounted with silver initial and 2 gold coins and shows shape of the interior.

No. 4 Cardboard case silver bicycle warrant.

No. 5 Wooden box of which the grain is plainly seen, a silver coin and gold ring.

No. 2 copy is a hand with cut finger."

The last item is of the greatest interest as it records the first known instance of an X-ray photograph with a medical slant produced in Malta.

It appears that the results obtained by Mr. Ellis were sufficiently satisfactory as to induce him to offer his services to the Military Hospital at Valletta. On the 17th November 1896 he wrote thus to Surgeon Major General Thomas Maunsell C.B.:

Sir,

I have been studying from its first appearance the production of pictures by the Röntgen Rays which if I could find encouragement and assistance I will continue its way — I am sending you some samples of my work to see the result of my applications. If you deem my services useful in the Military Hospital I will be greatly obliged if you would kindly lend me your assistance.

Tendering my excuses for taking such liberty.

With anticipated thanks,
I remain Sir yours,
John Ellis."

The Chief Secretary to Government replied in the following terms:

"4785/E 11th December 1896

Sir,

With reference to your communication received on the 3rd ultimo submitting results of photographic experiments taken with Röntgen rays, I am directed by His Excellency the Governor to acquaint you that information has been given to the Department concerned with a view to its being known that your services would be available when skiagraphy may be required at the Civil Central Hospital in cases of difficult diagnosis.

I have the honour to be,
Sir,
Your obedient servant
G. Strickland,
Chief Secretary to Government."
(Letter Book, 1896).

It is not possible to follow up the sequence of these letters as none of the Strickland and Ellis papers for 1896 are

extant (Ellis, 1971; Strickland, 1971).

Mr. John Ellis (1866-1931) eventually succeeded his father Richard as head of the photographic firm. He became a leading member of the commercial community in Malta, one time President of the Society of Arts, Manufactures and Commerce and an *Officier de l'Academie Francaise*. (The Daily Malta Chronicle, 1931).

Surgeon Major General Maunsell was in the British Army from 1860 until 1899. It is not known whether he passed on Mr. Ellis's letter to the army medical authorities in the United Kingdom but a search in the archives of the RAMC Historical Museum reveals no records relating to Mr. Ellis's communication (MacLennon, 1971).

Two years later the Maltese public had the first opportunity to hear a lecture on X-rays and witness their practical application. The occasion was provided by the so-called Lyceum Popular Lectures which the Director of Education organised during the winter. The first lecture in the series of the season 1898-99 was held in the Great Hall of the Lyceum, Merchants Street, Valletta, on the 3rd December 1898 under the Chairmanship of the Director of Education, Professor Napoleon Tagliaferro.

The title was "The Photography of the Invisible"; the lecturer was Dr. Themistocles Zammit. He illustrated the talk by means of the "optical lantern" and produced and demonstrated the new rays by means of an apparatus assembled by himself.

Zammit began by informing his audience that light was the result of the "very rapid vibration of matter exciting a series of minute waves in the ether". These waves travelled with a velocity of about 186,000 miles per second and were so small that as many as fifty thousand of them went to the inch. They could be produced by different means including electricity. Thus if an electric current was passed through a Geissler tube, containing gases or air in a rarefied state, it gave rise to "astonishing and beautiful luminous and colour effects". If, on the other hand, strong electric sparks from a special kind of induction coil were passed through a Crooke's tube, in which exhaustion had been carried to a very high degree, the new light rays were produced.

Zammit went on to explain how these rays were capable of penetrating certain opaque substances like wood, leather, clothing, some metals and also muscular tissue with as much ease as ordinary light passed through a plate of common glass. These X-rays could be brought within the perception of the sense of sight by means of the interposition of a luminescent screen made of paper coated with platinocyanide of barium which caught the shadow, cast by the rays, of the objects under observation.

The lecturer told how Röntgen, in his investigations of X-rays, discovered that a photographic plate was affected by the rays and that it was, therefore, possible to use photography to make a permanent pictorial record of what the rays rendered visible to the eye of the investigator.

Zammit then demonstrated the penetrative powers of X-rays by means of his apparatus and of a screen made of a piece of stiff paper covered with platino-cyanide of barium. Dr. Zammit did not actually "photograph the invisible", wrote a reporter, "but he placed a pair of scissors between the side of a wooden box and a book of two hundred pages and showed by means of his tube, full of an uncanny ghostly green fluorescence produced by crackling sparks of electricity, and his screen how such could be effected without much trouble. He likewise revealed the contents of a purse borrowed from one of the audience; he rendered visible the bones of his own hand and arm; he showed his watch that had been placed inside a box and exhibited the electric spark passing through air and Geissler tubes containing different gases."

Another member of the audience recorded that as these experiments were necessarily carried out in the dark and as the lecture hall was rather large, only a small portion of the audience "enjoyed the pleasure of seeing the marvels revealed". When, however, Zammit switched on his "magic lantern" in the second part of his demonstration, "every one could see

Fig. 1: The earliest dated X-ray photograph known, so far, to have been produced in Malta. It was taken by Professor Themistocles Zammit and is signed and dated by him Sept. 96. Very probably it is an X-ray picture of his own hand.
(Courtesy of Capt. C. Zammit, Malta)

what was described for the pictures were large enough and clear enough. They consisted of a portrait of Professor Röntgen, the discoverer of the wonderful light, and photographs of various objects actually taken by its action; for example a pair of spectacles within a leather case—curiously enough glass is rather opaque to the rays;—a razor blade seen through its case and handle; the bones of a foot, of several hands and of a boy's thorax in which a half-penny was clearly discernible. Those who could follow Dr. Zammit in his very instructive lecture could not fail to understand the use to which the rays could be put in all kinds of surgical examinations of the human body, by Customs Officers examining parcels as well as in all other observations of a similar character" (The Malta Times, 1898; the Daily Malta Chronicle, 1898; *Il portafoglio maltese,* 1898).

In the X-rays experiments Zammit was assisted by Dr. Thomas Agius and in the projections by the "optical lantern" by Dr. Daniel Marguerat.

The audience, "considering the inclement weather, was fairly large". The lec-

ture and demonstrations were judged to have been "highly successful and most interesting and instructive" especially to those "who had the good fortune to occupy seats in the half-dozen rows in the immediate vicinity of the lecturer's table" (The Daily Malta Chronicle, 1898).

Dr. Zammit was asked to repeat the lecture as, on account of the bad weather, many persons could not attend it. It appears that he had delivered the original talk in Italian as the advertisement announcing the repeat lecture stated that it would be given in English *(Il risorgimento,* 1898a; The Daily Malta Chronicle, 1899). It was eventually held on the 21st January 1899 in the Drawing Hall of the Lyceum.

Before proceeding further it may not be amiss to give a bare outline of the career of the three men who set up these lecture-demonstrations.

Dr. Themistocles Zammit (1864-1935) (later Sir Themistocles) held the post of Analyst in the Health Department at the time of the X-rays experiments. In 1905 he discovered the *Micrococcus melitensis* in the blood of the goat and thus showed that Undulant Fever was transmitted to man from infected goat's milk. In the same year he was appointed to the Chair of Chemistry at our University and in 1920 was chosen as its Rector. He was a world renowned archaeologist. He left medicine to become the first Director of our National Museum (Mifsud Bonnici, 1960-68a).

Dr. Thomas Agius (1871-1934) qualified in medicine and surgery in 1895 and, at the time he helped Dr. Zammit, he was Assistant Medical Officer at the Central Hospital (1896-99). He subsequently became Professor of Physics (1902) and Officer in Charge of the Meteorological Observatory at the University and finally Rector in 1926 (Mifsud Bonnici 1960-68b).

Dr. Daniel Marguerat (1873-1960), after obtaining the M.D. at our University, was appointed dissector at the Anatomical Theatre and later District Medical Officer and Superintendent of the Hospital for Infectious Diseases at Gozo. He took an active part in the eradication of trachoma (Mifsud Bonnici, 1960-68c) and in advocating a change in the nomenclature of *Malta Fever* to *Brucellosis.* After his retirement from government service he became interested in international politics and especially in the question of disarmament (Marguerat, 1972).

Zammit's pioneering efforts prompted the government of the day to acquire an X-ray apparatus for the Central Hospital at Floriana which was then the general hospital of the Island *(Il risorgimento,* 1898 b). It was ordered by the Office of the Charitable Institutions from England in May 1899 (Cassar, 1965).

The British Government sought to stimulate interest in the medical application of X-rays among practitioners in British possessions overseas. On the 8th May 1901 a circular from Downing Street invited medical officers from the various colonies on leave in England to avail themselves of the offer, made freely by the Society of Apothecaries of London, to attend demonstrations organised by the Society of the workings of the Röntgen Ray apparatus (The Malta Government Gazette, 1901). I have, however, found no evidence that any Maltese doctor attended these demonstrations (Busby, 1971; Public Record Office, 1971).

Prof. P. P. Debono states that the first X-rays equipment was installed at the Central Hospital about the year 1908, the apparatus being housed in a room on the right immediately on entering the hospital.

The first medical man to be entrusted with the working of the one and only X-rays plant in a government hospital seems to have been Dr. Francis Jaccarini (1884-1935). He graduated M.D. from the course of medicine and surgery of our University (1903-1907) in September 1907 after obtaining the highest number of marks in the quarterly and annual examinations and gaining the Government Exhibition Prize of £120 as First Student in that course.

In October of the same year (1907) he proceeded to London where he attended for three months the X-rays Department of Charing Cross Hospital and the Electric Department of the Hospital for Sick Children in Great Ormond Street. During

this period of training he was assistant to Dr. William Ironside Bruce, Physician and Radiographer to the two departments mentioned and author of "A System of Radiology with an Atlas of the Normal" (Bruce, 1907).

On his return to Malta, Dr. Jaccarini was appointed Assistant Medical Officer at the Central Hospital on the 1st October 1908. Apart from the yearly salary of £50 attached to this post he also received an allowance of £10 annually as "officer in charge of electrical apparatus" (Malta Blue Book, 1910).

During the earthquake which shook Sicily and Calabria in December 1908, he was among the group of Maltese doctors who went to the aid of the stricken population and was awarded the commemorative Silver Medal of the Italian Red Cross and the title of *Uffiziale dell'Ordine della Corona d'Italia* in recognition of his services (Jaccarini, 1972). During his brief career as radiologist, his guide was his master's *Atlas* acquired in London in 1907 and bearing Brace's signature. He was appointed District Medical Officer in 1910 (27th July) (Malta Blue Book, 1911a) in which capacity he spent his short professional life dying at 51 years of age on the 15th August 1935.

He was followed in 1910 by Dr. Salvatore Borg (1881-1943), later Professor of Physics and Officer in charge of the Meteorological Observatory at our University (Malta Blue Book, 1911b).

He was succeeded by Dr. Peter Xuereb (1886-1963) who qualified Doctor in Medicine and Surgery in 1910. He was Medical Officer in charge of the X-rays Room at the Central Hospital Floriana, during his appointment as Assistant Resident Medical Officer, from 1911 to 1914 (Malta Blue Book, 1912). During World War I he was attached to the RAMC as Civil Surgeon. In 1920 he went to London where he did Clinical Clerking under Sir Archibald Garrod, then Consultant Physician at St. Bartholomew's Hospital. In 1926 he was appointed Professor of Physiology (1926-39). His interest in X-rays had not waned for while attending the Vienna General Policlinic in 1929 he took the opportunity to acquaint himself with the radiological diagnostic methods then available (Xuereb, 1938). He reached the top rung of his career when he became Professor of Medicine (1939-1946). He died in 1963.

The next incumbent was Dr. Joseph

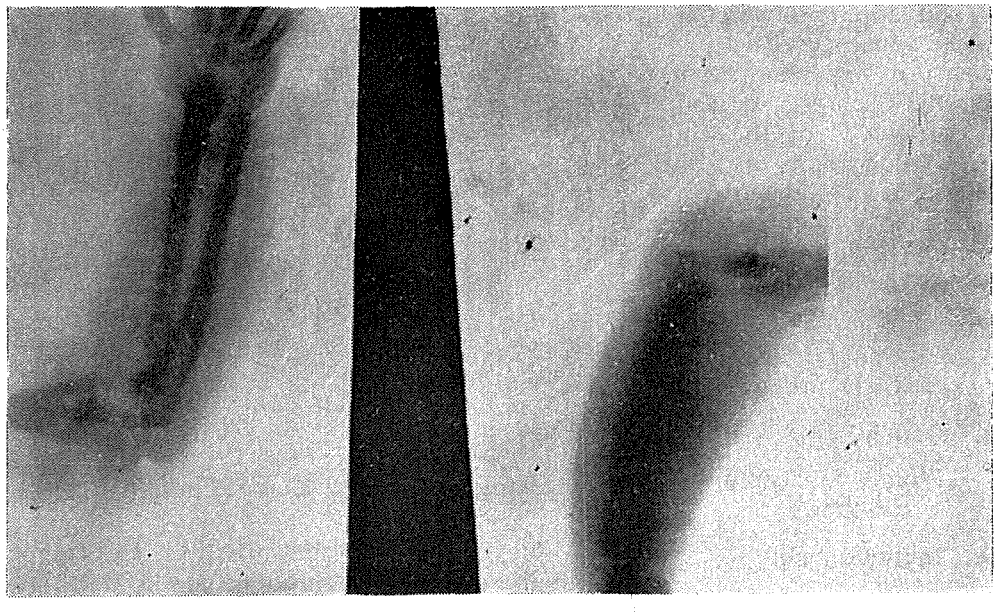


Fig. 2: The earliest diagnostic X-ray photograph extant taken by Dr. F. Jaccarini (1908-10). *(Courtesy of Dr. J. and Miss R. Jaccarini).*

Ellul, later Professor of Midwifery at our University (1930-1951). Born in 1888, he qualified in 1913. On the 1st October 1914, he was appointed Assistant Resident Medical Officer at the Central Hospital and also Medical Officer in charge of the Electric Room and Radiographer. He was concurrently attached to the Royal Army Medical Corps as Civil Surgeon and in 1916-17 he became Voluntary Assistant to the Radiographer at the Tignè Military and at Bighi Naval Hospitals. He gave up his post at the Central Hospital on the 30th September 1917 (Ellul, 1930).

The X-rays apparatus used by these early workers was "very primitive in type and not very efficient" (Debono, 1947). No parts of this apparatus have survived the ravages of time but we can form an idea of what it looked like from contemporary illustrations and descriptions of similar equipment elsewhere. The patient was placed upon a couch consisting of a wooden frame covered with canvas. The X-ray tube was contained in a box that moved on wheels and rails beneath the couch. It was lined with metal to shield the operator fro the X-rays. The electric current was obtained by an induction coil. A switch board, and amperemeter and a plumbline completed the apparatus. The time of exposure depended upon the strength of the current used, the power of the coil and the condition of the Crookes tube, i.e. whether a high or a low vacuum one. The accompanying illustration shows the component parts, viz.

A. Box containing the X-ray tube

B. Couch

C. Plumb for centering the tube

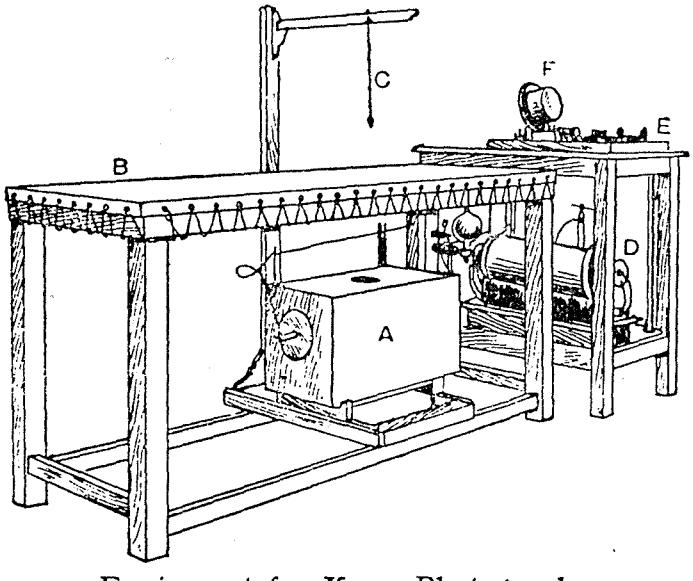
D. Induction coil

E. Switchboard

F. Amperemeter (Cassell, 1911).

With the outbreak of World War I in 1914 and the consequent establishment of the many military hospitals that sprung up all over the Island, a number of X-ray sets were brought to Malta and set up at Valletta, St. Andrew's, Cottonera, Tignè and St. Ignatius Hospitals (Bruce, n.d.). Contemporary photographs of two apparata of the time are extant. There are two views of a stand for the tube and plate with a support for the head for taking X-rays of the skull. The manufacturer has not been identified (Poynter, 1971).

The other photograph shows the Mackenzie-Davidson Localiser for the accurate determination of the position of foreign bodies probably dating from late 1914. This apparatus and its method of use are described in E. B. Morton's *Textbook of Radiology* (Morton, 1918). "The Mackenzie-Davidson method of localisation is a very accurate one... The actual taking of the radiograph is very much the same as for stereoscopic work, except that the two exposures are made on one plate — and not on separate plates. The plate in its wrapper is tied up with a piece of



Equipment for X-ray Photography

Fig. 3: X-ray equipment of 1911 contemporary with the earliest apparatus in use in Malta at the beginning of this century. *(By kind permission of Bailliere Tindall, London, from Cassell's Cyclopaedia of Photography, London, 1911, p. 569).*

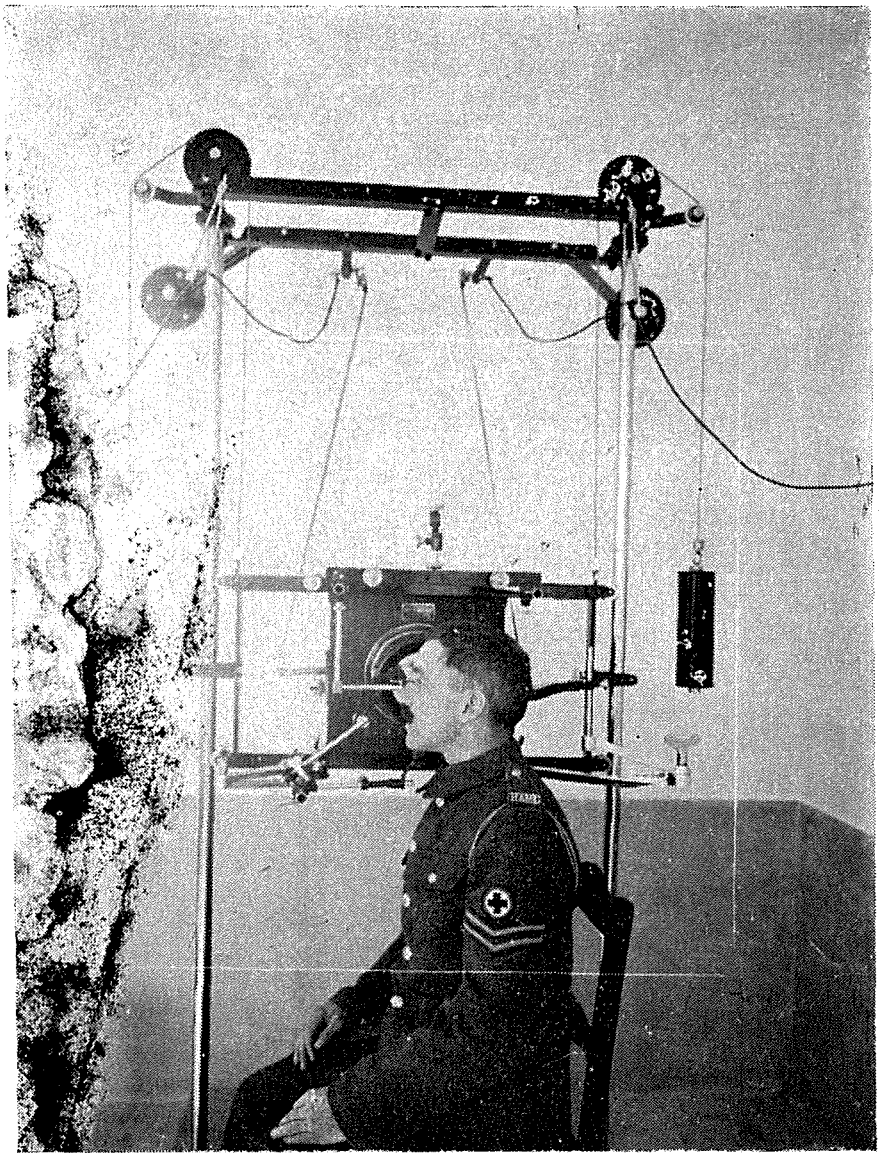


Fig. 4: Stand for the tube and plate with a support for the head for X-raying the skull in use by army medical units in Malta during World War I. *(Courtesy of Mr. Richard Ellis, Valletta).*

fine wire just as we would tie up a parcel. The wires where they cross in the middle of the front of the plate are smeared with aniline ink. The position of the foreign body having been first ascertained approximately by the screen, the plate is laid against the skin and underneath the part. The tube is arranged with the anti-cathode vertically over this point and the two exposures made — each about three centimetres on either side of the centre. On removing and developing the plate we have an aniline mark on the skin and a corresponding cross on the plate. We have also two shadows on the plate thrown by the foreign body with the tube in its two positions. The plate is now placed centrally on the Mackenzie-

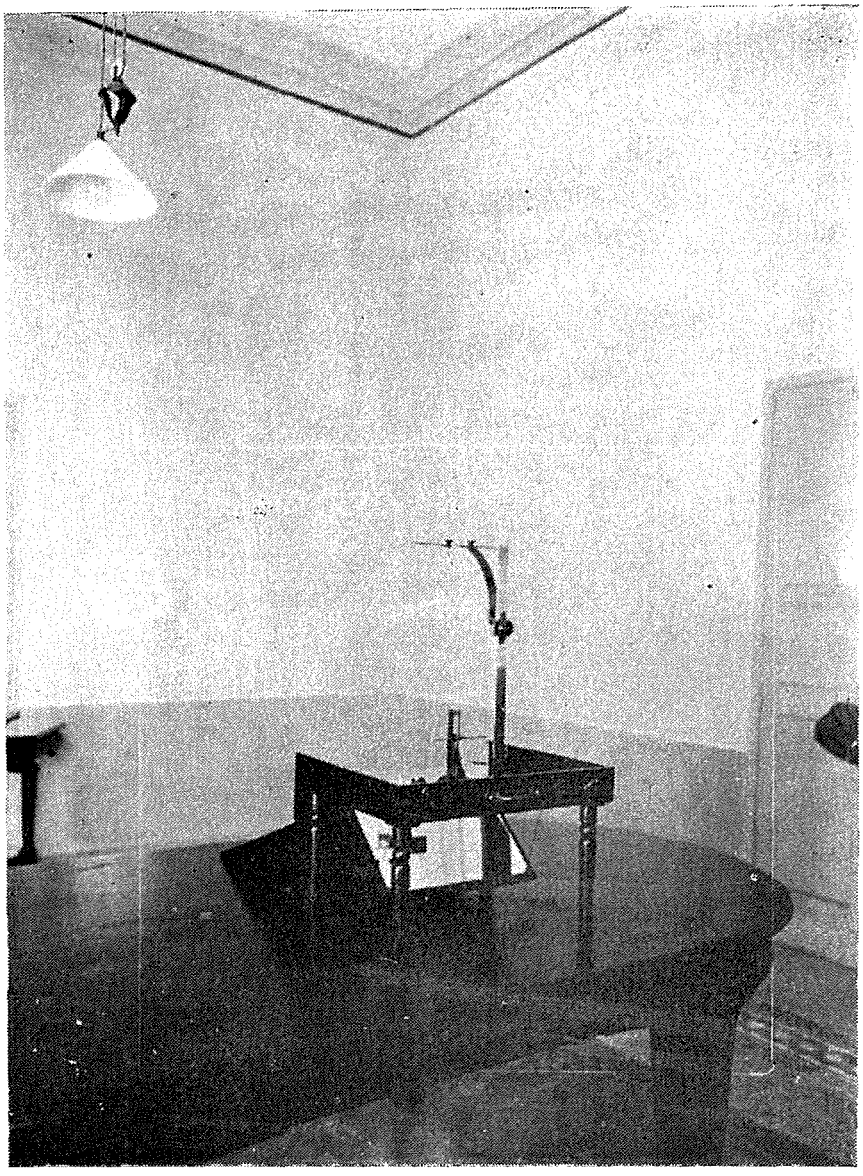


Fig. 5: The Mackenzie-Davidson Localiser for accurate determination of the position of foreign bodies employed by army medical units in Malta during World War I. *(Courtesy of Mr. Richard Ellis, Valletta).*

Davidson localiser, which consists of a plate glass table with two uprights and a graduated crossbar sliding thereon. The crossbar is set at the same height as the anti-cathode was from the plate when the exposures were made. Two threads with small weights at each end to keep them taught are brought over the crossbar at points three centimetres from the middle line and led to the centre of the shadow of the foreign body of the opposite side so that the two threads cross at a certain point. The threads represent the central ray when the images were photographed, and, of course, will cross at the position of the foreign body. The height and horizontal distance from the central cross are then measured with calipers. With these

two distances and the mark on the patient's skin, the foreign body can be easily found in most cases."

By the time some of the military hospitals were closed down at the end of the war, the Maltese Government came to realise that the X-ray apparatus at the Central Hospital, which had been obtained "many years previously", had become inadequate. Government, therefore, took the opportunity of acquiring the X-rays apparatus of Tignè Military Hospital for £300, "a price much lower than what government would have had to pay if the apparatus were to be purchased from abroad". At the sitting of the Council of Government of the 5th July 1919, the Lieutenant Governor stated that the apparatus was a "very excellent and efficient one" and that the medical and surgical staff of the Central Hospital had strongly recommended its acquisition without delay (Debates Council of Government, 1923; The Malta Government Gazette, 1919).

Dr. Joseph Ellul was followed by Dr. Joseph Briffa in 1919. Dr. Joseph Briffa was born at Valletta on the 25th February 1891. He matriculated in 1909, obtained the diploma of Pharmaceutical Chemist in 1912, the B.Sc. in 1913 and the M.D. in 1916 (2nd October). During the whole academic course (1912-16) he had obtained the highest number of marks in both the Term and Annual Examinations and was, consequently, awarded four Government Scholarships entitling him to exemption from school and examination fees. At the end of the course he was declared the First Student of the Course and, as such, awarded the University Exhibition of £120 and the Marquis Bugeja Prize of £50.

Soon after graduation he proceeded to the University of Naples and joined the *Istituto di Clinica Medica* directed by Professor A. Cardarelli whose lectures he followed during the years 1916-17. He also attended several courses of studies in Clinical Surgery, Diseases of Children and Ophthalmology.

Between the 1st June and the 11th September he followed a practical course in Diagnostic Radiology at the *Istituto Röntgen* of the same University under its director Dr. Pasquale Tandoia (1870-1934), one of the most distinguished Italian radiologists of the time (Castiglioni, 1948). In a testimonial Dr. Tandoja stated that Dr. Briffa was excellently prepared academically so much so that he was able "to obtain, in a short time, the greatest benefit from his study of this speciality".

On his return to Malta in October 1917, he was appointed Assistant Resident Medical Officer at the Central Hospital. He relinquished this post on the 10th June 1919 when he was appointed Radiographer and Medical Officer in charge of the Electrical Room at the Central Hospital. In his new capacity he was instrumental in modernising the apparatus at the Central Hospital and thus laid the foundations of a proper X-rays Department at that Hospital.

In those days photographic glass plates were used instead of the present celluloid films which came into use in Malta about 1928. The glass plates were obtained from, and developed by, the firm of Chretien & Co. of the Empire Studio, 291, Kingsway, Valletta, the site now occupied by Kingsway Pharmacy near St. Francis Church. They were preserved for five years and then destroyed. None of these glass plates appear to have survived.

By 1924 the "increasing demand for X-ray examinations and X-ray treatment" necessitated the expansion of the department. Structural alterations were effected and additional accommodation was provided for the setting up of a plant for treatment by diathermy and high frequency current (Report of Comptroller of Charitable Institutions, 1926).

The protective measures then available against the harmful effects of the rays consisted in the wearing of eye-shields and of rubber gloves and aprons; but the gloves were so unwieldy and the aprons so heavy that both Dr. Briffa and his assistant, Dr. J. M. Sammut, preferred to do without them as they hampered their movements. A partition lined with lead sheeting and provided with an observation window closed by a lead glass pane ,separated the patient's cubicle from that of the radiographer. Protection of

patient and radiographer was brought up-to-date by 1930 (Sammut, 1971).

A change in nomenclature to Radiologist and Medical Officer in charge of the Electrical Room was effected on the 1st April 1937; and to Radiologist on the 1st January 1947.

In 1920 Dr. Briffa held the post of Demonstrator in Anatomy at the University and, while still working as radiologist, was appointed to the Chair of Anatomy and Histology in 1939. Three years previously (1936) he had been appointed to teach radiology to dental students by the General Council of the University.

His experience of radiology had placed him in constant touch with anatomy in so far as his X-ray practice involved frequent references to the structure of the skeleton and the various organs that lent themselves to radiological examination. As Professor of Anatomy he will be remembered for having replaced the teaching of Systemic by that of Regional Anatomy and for introducing the study of Embryology in the medical curriculum.

When he came back to Malta from Naples in 1917 he had gone into general practice but later set up a private radiological clinic at No. 6 Merchants Street, Valletta, in the vicinity of the Health Department Office. I have not yet found out when the clinic was first opened but it is on record that it was functioning before 1930. This was the first private radiological clinic to be established in Malta.

Sometime in 1939/40 he bought new apparatus and moved to a room at the Dominion Pharmacy which was sited at the corner of Kingsway with St. John Street now occupied by the National Bank of Malta and other business premises. When this part of Kingsway was destroyed by air bombardment during World War II, he lost all his equipment and he gave up private practice.

Dr. Briffa retired from the government service on the 24th December 1951 on reaching the age of sixty years but continued to hold the Chair of Anatomy until 1952 (The Royal University of Malta Calendar, 1958; Briffa Boothman, 1972; Cuschieri, 1972; Times of Malta, 1959).

Prof. Briffa's successor was Dr. Joseph Sammut (1895-1972). He began his studies ot the Royal University of Malta (1912-18) but completed them at the *Regia Università di Napoli* where he graduated M.D. in June 1920 after studying medicine under Prof. A. Cardarelli, clinical medicine under Prof. G. Moscati and psychiatry under Prof. L. Bianchi.

On his return to Malta he was honorary assistant to Dr. A. Portelli Carbone, *interim* professor of Medcine, at the Central Hospital (1920-24). It was during this period that Dr. Sammut became interested in X-rays. In 1924 he began to attend regularly the X-rays Department carrying out radiological examinations and radiotherapy under the direction of Prof. Briffa.

In 1926 he went back to Naples to study radiology under Prof. Felice Piccinino at the *Santa Casa degli Incurabili ed Ospedali Uniti* and under Prof. C. Guarini of the *Istituto-Foto-Radio-Terapico (Policlinico)*. On the 4th October of the same year he was appointed Assistant Radiologist and Medical Officer in charge of the Electrical Room at the Central Hospital (the Malta Government Gazette, 1926).

At the outbreak of World War II in June 1939 he was entrusted with the organisation of the X-rays Department set up at the Bugeja Emergency Hospital at Hamrun. He directed this department during the war years (1939-45). In the meantime he contributed to the initial training of his two successors — Dr. F. Callus and Dr. F. Zammit who were his assistants in 1938-39 and in 1942-43 respectively.

In 1944 he was awarded a scholarship by the Trustees of the Nuffield Foundation for a year's course in radiology in the United Kingdom which he actually followed in 1946-47 at the Regional Radium Institute of the Royal Infirmary at Bradford (Dr. F. Chester Williams), the Liverpool Radium Institute (Dr. J. S. Fulton) and the Leeds General Infirmary (Dr. A. Johnstone). In 1947 he was appointed Radiologist at St. Luke's Hospital (15th April), a post which he occupied until his retirement on the 3rd March 1955 on reaching the age limit in the government service. He died on the 4th August 1972

(Sammut, 1972; The Malta Government Gazette, 1947).

With the retirement of Dr. Joseph Sammut we come to the end of this survey of the first threequarters of a century of radiology in Malta. This period embraces three phases:

1. The experimental phase of X-rays from 1896 to 1908 represented by the pioneering efforts of Mr. John Ellis and Dr. Themistocles Zammit.

2. The introduction of medical X-rays at the Central Hospital in 1908. During this stage, which lasted until 1919, radiology was still undifferentiated from other branches of medical practice and was considered to be well within the competence of the newly-fledged doctor with little or no *ad hoc* training and experience. It is represented by the work of Dr. Francis Jaccarini, Dr. (later Professor) Salvatore Borg, Dr. (later Professor) Peter Xuereb and Dr. (later Professor) Joseph Ellul.

3. The third phase marks the recognition in 1919 of medical radiology as a distinct speciality in its own right to be entrusted to a specially trained practitioner. This stage, initially represented by Dr. (later Professor) Joseph Briffa and by Dr. Joseph Sammut, subsequently evolved into the expansion of contemporary radiology in Malta.

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THYROTOXICOSIS IN PREGNANCY

VANNI CREMONA
M.D., M.R.C.O.G.

Thyroid function is closely interrelated with the development and function of the female genital organs. Myxoedema and cretinism delay the onset of menstruation and the development of secondary sexual characteristics. When menstruation is present it tends to be irregular and infrequent. In adult hypothyroidism menorrhagia may occur, while with hyperthyroidism oligomenorrhoea or amenorrhoea frequently occur but menorrhagia is rare. The thyroid gland is affected by changes in reproductive function. Thyroid enlargement is six times as frequent in the postadolescent woman as in man. It is apt to occur at puberty, in pregnancy and at the menopause. Oestrogens are known to affect numerous parameters of thyroid function. Probably owing to an increase in thyroid binding globulin (TBG) the protein bound iodine (PBI) is raised and the radioactive triodothyronin ($^{131}T_3$) red cell or resin uptake is decreased .This fact is of major clinical importance as the contraceptive pill or other oestrogen therapy may cause errors of judgement in the evaluation of thyroid function tests in cases of suspected throid dysfunction.

Fertility is also affected by changes in thyroid function. Sterility is common in myxoedema although instances of pregnancy in severe untreated hypothyroidism have been reported. When pregnancy results, abortion and stillbirths are common. Even when the pregnancy is carried successfully to term, congenital goitre may occur. Moreover a high incidence of developmental abnormalities is seen in euthyroid offspring of hypothryoid mothers. Untreated thyrotoxicosis is also associated with a decreased fertility rate. Keynes (1952) reported a fertility rate of 52.5% in thyrotoxic women before operation compared with a normal rate of 70%.

Thyrotoxicosis is a disease of unknown aetiology with a striking genetic or familial incidence. The condition affects women more often than men and is predominantly a disease of the childbearing era. In spite of this it is rarely found in association with pregnancy. The incidence in reported series varies widely. Silver (1965) comparing the incidence of several series found that the overall mean incidence of thyrotoxicosis in pregnancy is 0.047%.

Before considering the treatment of thyrotoxicosis in pregnancy it is important to determine what effect thyrotoxicosis has on pregnancy, the changes in thyroid function that occur in normal pregnancy and the effect of pregnancy on thyrotoxicosis.

Effect of Thyrotoxicosis on Pregnancy

There is general agreement that uncontrolled thyrotoxicosis predisposes to

abortion, premature delivery and stillbirth and that the risks are proportionate to the severity of the thyrotoxicosis. Proper control eliminates most of these risks as is seen in most modern series. In the past the incidence of pre-eclampsia seems to have been very high in pregnancy complicated by thyrotoxicosis. McLaughin and McGoogen (1943) reported an incidence of 62% in their series of only 19 cases. In contradistinction in Hawe and Francis' series of 70 patients only 4 (5.7%) developed pre-eclampsia. With modern treatment this complication does not occur more commonly than in euthyroid pregnant women.

The foetus is not usually affected by thyrotoxicosis, but rare cases of congenital thyrotoxicosis or exophthalmos have been reported. The condition is usually benign and settles spontaneously or with minimal treatment.

Effect of pregnancy on thyroid function

Most of the symptoms and signs associated with hyperthyroidism are present in a lesser or greater degree in pregnancy.

Many clinical features are common to pregnancy and to thyrotoxicosis. These include increased cardiac action, accelerated peripheral circulation, and decreased heat tolerance. The thyroid gland often enlarges during pregnancy. This was recognised by the ancient Egyptians who used it as a test for pregnancy by tying the stem of a plant round a woman's neck. Pregnancy caused the stem to break. Few well controlled trials into the incidence of goitre in pregnancy have been reported. Crook *et al.* (1964) reporting from Aberdeen found an incidence of 70% in pregnant women against an incidence of 37% in non--pregnant controls. They also noted that the incidence was constant throughout pregnancy.

There are very few reports on the histological changes in the thyroid gland during normal pregnancy. Stoffler (1957) studied the thyroid glands of 65 pregnant women at autopsy and reviewed a further 28 cases from the literature. He found no evidence of increased thyroid weight and suggested that any increase in size must be due to hyperaemia. The histological picture of enlarged follicles full of thin colloid with vacuolisation and an epithelium which is generally hypertrophic led him to conclude that there is increased activity of the thyroid gland during pregnancy.

The basal metabolic rate is increased during pregnancy. The rise is apparent after the fourth or fifth month and gradually rises until term when it is raised about 20% above non-pregnant levels. Attemps to correlate this phenomenon with changes have failed.

TABLE I

Determination	*Non-pregnant normal*	*Pregnant normal*
PBI	4.0–7.5 μg%	6.5–11.5 μg%
BEI	3.2–6.5 μg%	5.5–10.5 μg%
T_3 RBC uptake	11–17%	< 10%
T_3 Resin uptake	25–30%	< 10%
Free thyroxine factor	0.384 (±0.0843)	0.375 (±0.103)
Free thyroxine	1.4–2.5 mμg%	1.4–2.5 mμg%

Normal values for thyroid function tests in pregnancy.

The increased oxygen uptake in pregnancy is probably due to an increase in protoplasmic mass and the increased cardiac and respiratory work which occurs during pregnancy.

PBI also rises early in pregnancy to levels consistent with hyperthyroid states. Increased levels are found as early as 3 to 6 weeks of gestation. Man (1961) reported that the levels are consistently raised by the 16th week of pregnancy. Butanol extractable iodine (BEI) which reflects the total serum thyroxine level shows a similar increase in the binding capacity of the TBG either due to a quantitative increase in the protein or due to a qualitative alteration in its binding capacity. This increase in binding capacity is reflected by a decrease uptake of $^{131}T_3$ by red cells or resin which is seen as early as the fourth week of pregnancy. It is believed that the increase in thyroxine binding capacity, which accounts for the rise in PBI and BEI, is secondary to the effects of oestrogens. However, recent reports do not confirm this.

Radioactive I^{132} which has a half life of only 77 hours has been used to study thyroid activity during pregnancy. Halnan (1958) found that there is an increased uptake of iodine by the thyroid gland during pregnancy. In a later more extensive study by Aboul-Khair (1964), the plasma inorganic I level was found to be low throughout pregnancy. This is due to an increased renal clearance of I which is high throughout pregnancy. This causes a relative iodine deficiency in the immediate vicinity of the thyroid gland with the result that clearance of I by the thyroid is increased in order to maintain the absolute iodine uptake within euthyroid limits. These findings suggest that in normal pregnancy there occurs no increase in thyroid hormone production.

Recent advances in methods available for studying thyroid function have confirmed that normal pregnant women are euthyroid. Free thyroxine factor, which is based on a calculation between $^{131}T_3$ RBC or resin uptake of PBI levels and reflects the level of free thyroxine in the serum, shows no significant difference between normal pregnant and non-pregnant women. Free thyroxine estimations have also been performed and confirm these results. It seems therefore, that the usual tests of thyroid function are distorted by the metabolic and cardiovascular changes which occur during pregnancy and that any enlargement of the thyroid gland is due to the relative iodine deficiency.

These metabolic and cardiovascular changes make the diagnosis of thyrotoxicosis during pregnancy difficult. As we have seen before, certain clinical features of pregnancy and thyrotoxicosis are remarkably similar. However, the more marked these clinical features, the more likely is the probability of co-existing thyrotoxicosis, especially a persistently high sleeping pulse rate, failure to gain weight despite good appetite, excessive sweating, tremor, and a considerably enlarged pulsatile thyroid gland. The presence of exophthalmos may provide valuable evidence as does a history of symptoms which preceded the onset of pregnancy. Laboratory aids are not usually helpful especially in mild cases. The use of I^{131} is contraindicated, but I^{132} may be useful in exceptional circumstances. Halman calculated that with a dose of 6 μc of I^{132} the foetal thyroid is exposed to only 0.01 to 0.06 rads. and the foetal and maternal gonads to less than 0.001 rads. $^{131}T_3$ uptake studies and especially the calculation of free thyroxine factor appear to reflect the true state of thyroid function and may prove to be the best guide as to the severity of the thyrotoxicosis and to the level of control during treatment.

Effects of pregnancy on thyrotoxicosis

Thyrotoxicosis often shows spontaneous changes in severity and this makes interpretation of the effect of pregnancy difficult to evaluate. There is no general agreement, the camp being equally divided between those who consider that adverse effects may occur and those who maintain that pregnancy has a favourable effect on thyrotoxicosis. However, as Hawe and Francis remark there is an increased risk of thyroid crisis during or immediately after delivery.

Treatment of thyrotoxicosis during pregnancy

During pregnancy two plans of treatment are available for the control of thyrotoxicosis. One is to control the hyperthyroidism with antithyroid drugs throughout pregnancy. The alternative is to perform subtotal thyroidectomy after controlling the disease with a short course of antithyroid drugs or iodine. Radioactive iodine which is the method of choice in patients over 45 years of age, is absolutely contraindicated during pregnancy. The isotope crosses the placental barrier and is accumulated with great avidity by the foetal thyroid after the first trimester. The risk of damage to the foetus is very real especially as the thyroid glands of infants are known to be unusually sensitive to radioiodine. Russell *et al.* (1957) reported two cases of severe hypothyroidism in infants whose mothers were given I^{131} during the 13th week of pregnancy.

Medical treatment

Control of thyrotoxicosis with antithyroid drugs throughout the pregnancy is usually considered as the treatment of choice. The dosage varies from patient to patient. The general aim is to obtain control of the hyperthyroid state and then reduce the dose to the minimal maintenance level. In judging the level of control one must always be aware of the apparently raised levels of thyroid function characteristic of normal pregnancy. The usual difficulties of controlling hyperthyroidism are enhanced during pregnancy. Over-treatment may endanger the foetus and under-treatment may lead to an exacerbation of maternal thyrotoxicosis; both situations are associated with an increased risk of foetal loss. In the interests of the foetus the usual practice is to reduce or stop antithyroid drugs during the latter months of pregnancy. Unfortunately due to occasional unexpected early relapses this is not always possible.

Antithyroid drugs are known to cross the placental barrier and depress the foetal thyroid gland. This results in increased production of thyroid stimulating hormone (TSH) and hyperplasia of the foetal gland. Foetal goitre is usually transient and subsides spontaneously; however, several instances of a large goitre leading to neonatal death from tracheal compression have been recorded and several cretins have resulted from the use of antithyroid drugs. Furthermore, antithyroid drugs are excreted in the milk and therefore breast feeding is contraindicated during their use.

In spite of these difficulties some excellent series have been reported. Astwood (1951) treated 19 patients with propylthiouracil or Carbimazole, substantially reducing the dosage during the second half of pregnancy. He obtained 22 live infants from 22 pregnancies and no foetal goitres. Piper and Rosen (1954) following a similar regime recorded four abortions and one stillbirth among 16 pregnancies. They reviewed the literature and were able to find records of 83 pregnancies during which antithyroid drugs were used to control hyperthyroidism, with a foetal loss of 6. Of the 77 viable infants 14 had goitres. Similar difficulties were experienced by Becker and Suddith (1959) and Hawe and Francis (1962). However, Herbert (1965) reported a series of 32 pregnancies during which antithyroid drugs were used following the usual regime, adding USP thyroid 120-180 mg. when the thyrotoxicosis was under control. They reported a foetal loss of only three and no instance of foetal goitre. The addition of thyroid hormone may be useful in avoiding maternal hypothyroidism, but it is doubtful what beneficial effects this has on the foetus as thyroxine crosses the placental barrier very slowly (Myant 1958).

Table II summarises the results of several series where antithyroid drugs were used to control thyrotoxicosis in pregnancy. Out of a total of 245 pregnancies, the foetal loss was 33 (13.5%); two of these were neonatal deaths due to a suffocative goitre. Of the 212 live infants 23 had goitres and 2 were cretins, an incidence of goitre of 9%.

Surgery

Subtotal thyroidectomy has been performed for the control of thyrotoxicosis

TABLE II

Author	*No. pregs.*	*Foetal loss*	*Viable infants*	*No. goitres*
Series prior to 1954 according to Piper and Rosen	83	6	77	15
Piper and Rosen (1954)	16	5	11	0
Becker and Suddith (1959)	34	9	25	2 1 cretin
Hawe and Frances (1962)	39	5	34	1 cretin
Burrow (1965)	41	5	36	5
Herbst (1965)	32	3	29	0
TOTAL	245	33 (13.5%)	212	24 (9%)

Foetal loss and incidence of goitre in series treated by antithyroid drugs.

during pregnancy since the beginning of this century. Almost consistently good results have been obtained. The operation is usually performed during the 2nd trimester due to the possibility of causing miscarriage if it is performed earlier. Even when performed during the first trimester instances of miscarriage following operation are rare. Prior to the introduction of the antithyroid drugs patients were prepared with iodine. Piper and Rosen (1954) collected 122 cases from the literature with a foetal loss of four. No foetal abnormalities occurred.

More recently antithyroid drugs have been used to obtain control of the hyperthyroidism as subtotal thyroidectomy is safer if performed on euthyroid patients. Lugol's iodine is given for 1-2 weeks prior to operation in order to diminish the increased vascularity of the thyroid gland which results from the use of antithyroid drugs. Following this regime most authors report good results. Bell and Hall (1960) suggest that the use of thyroid replacement therapy following operation is worthwhile as results seem to be greatly improved. They treated 21 pregnant patients with subtotal thyroidectomy following antithyroid drug therapy. In this group of patients there were 4 abortions and 1 stillbirth. Following this experience they treated a further 21 pregnant patients with a similar regime, but prescribed dessicated thyroid post-operatively until term. Only one infant, a stillbirth, was lost. Other authors (Hawe and Francis) have not confirmed this advantage of thyroid replacement therapy, but its use is justified as hypothyroidism may occur after surgery.

Table III summarises the results of several series where thyrotoxicosis was treated by subtotal thyroidectomy during pregnancy following control with iodine alone or with antithyroid drugs. Out of 300 pregnancies the foetal loss was only 22 (7.3%). No goitres occurred. Only one infant, a mongol, showed congenital abnormalities.

TABLE III

Author	No. Pregs.	Foetal loss	Viable infants
Collected series according to Piper and Rosen (1954)	122	4	118
Dailey and Benson (1952)	17	3	14
Stoffler (1957)	21	3	18
Holt (1959)	3	0	3
Becker and Suddith (1959)	8	1	7
Bell and Hall (1960)	42	6	36
Lange (1961)	31	1	30
Hawe (1965)	38	2	36
Hamilton (1968)	18	2	16
TOTAL	300	22 (7.3%)	278

Foetal loss in series treated with subtotal thyroidectomy.

Discussion

These figures provide good evidence of the safety of subtotal thyroidectomy for the mother and child. Occasional maternal deaths have occurred following surgery (Stoffler 1959), but maternal deaths have also been recorded with medical treatment (Hawe and Francis 1962). Although the numbers are small and not suitable for statistical analysis the rate of foetal loss and of neonatal mortality and morbidity are generally lower and more consistent following surgery, than with antithyroid drugs. It appears that operation during pregnancy does not increase the risk or adversely affect the foetus and, therefore, most of the pros and cons which affect the choice of treatment in young non-pregnant patients are valid during pregnancy.

At present the choice of treatment is largely determined by whether the patient is referred to a surgeon or a physician. Most physicians regard medical treatment as the method of choice in pregnancy (Crooke and Wayne 1960). With good medical care it is possible to obtain good results (Astwood 1951, Herbst 1965), but from time to time difficulties arise which may be outside the control of the clinician especially as treatment involves a compromise between the interests of the mother and of the foetus. Moreover the recurrence rate requiring subtotal thyroidectomy, following delivery, is high, (Piper and Rosen 1954, Becker and Suddith 1959, Hawe and Francis 1962).

Subtotal thyroidectomy does not have many of these disadvantages. Following operation the patient quickly returns to a euthyroid state, the pregnancy will then proceed as in any normal patient and breast feeding is not restricted. Moreover the recurrence rate after operation is low and subsequent pregnancies are not complicated by the persistence of the hyperthyroid state. There are certain qualifications which have to be fulfilled prior to undertaking surgery. The diagnosis, of course, must be assured; this avoids unne-

cessary operations on the mildly toxic patient in whom the diagnosis in pregnancy may be very difficult and who might recover spontaneously. The patient must be seen early in pregnancy as otherwise not enough time is available to make the patient euthyroid before operation. No contraindications to operation must be present.

In the final analysis the medical and surgical facilities available and the special circumstances of each individual patient will determine the management of a particular case. However, subtotal thyroidectomy performed by experienced surgeons appears to offer better prospects to the foetus and the mother.

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A COMMENT ON THE TIMES

(Continued from page 68)

the United States of America. In many ways we must see to it that Malta should become even more British than it has been, for the advantage it gives us in medicine apart from for a host of other reasons.

Should we make excuses for speaking of ethics and of sociology in a medical periodical? We do not think so since we believe that after all it is even more important to save a civilisation than it is to prolong a life for a few months or to indulge in the academic pleasure of describing some illness which affects only a few and perhaps only lightly. It all comes back to the same thing: we must foster our links because they are essential for the increase of our knowledge and this knowledge will serve us again to improve our medical learning. What we must not be is an island in any sense except the geographical one. No one in his senses will deny the value of contacts with every country, especially the historic countries of Europe which have civilised the world, but we must make the best of that accident which made us one with the country that is now what Athens was two thousand years ago.

MANAGEMENT OF THE CHILD WITH SPINA BIFIDA

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M.D., M.R.C.P., D.C.H.

During the period October 1965 to September 1966 I worked at Sheffield, in England, where a particular clinical interest in the problem of spina bifida exists. The following is based largely on the teachings of that school.

Meningomyelocele is one of the most important of the congenital anomalies with an incidence of about 3 per 1000 live births in Western Europe. In the management of the individual patient all aspects must be considered simultaneously and any one specialist involved in treating one particular side of the problem must be fully aware of the other associated conditions. A formidable list of the many problems that may present are shown in the figure. Here only the major ones will be discussed.

Meningomyelocele

The main aim in surgical repair of the spina bifida is to conserve as much neural function as possible. After birth the danger of infection is considerable. Also, the neural plaque dries out, with further damage to the nerves in the sac. It has been shown in Sheffield that the earlier the lesion is closed, the better are the results as far as leg movements are concerned (Sharrard *et al.* 1963). These infants are therefore dealt with as acute emergencies and are operated within 48 hours (preferably 24 hours) after birth.

One major difficulty for the paediatric surgeon is that it is virtually impossible to assess the potential of the newborn with this defect, so that selection for surgical treatment is also nearly impossible. Only very rarely can one conclude with certainty that the baby will not survive if given normal care. In most centres virtually all cases have up till recently been submitted to surgery, but this view has now been strongly challenged and some opinions are now in favour of some sort of selection of cases. Lorber in a recent paper has given a detailed account of the results of early surgical treatment of 524 unselected cases of meningomyelocele in Sheffield over the years 1959-63 and 1967 (Lorber 1971). In severely affected babies the overall results have been most disappointing and he concludes that those with extensive paralysis, gross hydrocephalus, severe kyphosis and those with major asociated congenital defects should not be operated upon. However, Eckstein still thinks that there are probably no absolute contraindications to surgery and each case should be treated individually (Eckstein 1972). The ethical problems which are raised by the birth of a severely deformed child have been widely discussed (Illingworth and Illingworth 1965).

While waiting for surgery the baby is nursed in an incubator and the spinal defect is covered with sterile saline-soaked gauze. An X-Ray of the whole body is taken and a bacteriological swab taken from the exposed neural plaque. On the operating table, a water blanket is used to maintain the body temperature and the operation area is isolated by means of 'Vidrape' which makes it possible for the surgeon to see the leg movements in response to electrical stimulation of various areas in the plaque. The operative procedure is straightforward. The neural plaque should be isolated and preserved but all membranous tissue should be excised leaving a clean skin edge. Extensive mobilisation of skin flaps by dissecting them off the deep fascia invariably destroys most of the skin blood supply. Good results, free from complications of wound breakdown, have been obtained by Rickham using a modification of Zachary's procedure in which the skin flaps are approximated by putting sutures through the dural strip which is left attached to the

skin placing the knots inside. The approximated skin edges are sutured loosely. If the vertebral laminae are very prominent they are nibbled off (Rickham 1969).

After operation the baby is nursed prone in an incubator, with a sling under the pelvis to relieve tension on the skin sutures. Any blood loss is made good by transfusion and antibiotic cover is provided. Leakage of C.S.F. may continue for some days but is rarely a persistent complication. The two major immediate complications of meningomyelocele are meningitis and hydrocephalus.

Meningitis is a grave neonatal emergency and on the least suspicion one must perform a ventricular tap and the C.S.F. must be examined so that the right antibiotic is given, based on sensitivities. Unfortunately, prophylactic antibiotics do not prevent meningitis; on the contrary they often lead to infections with organisms which are difficult to eradicate. It is imperative to give daily injections not only systemically but also into the cerebral ventricles, the initial antibiotic being one of the newer ones e.g.: Ampicillin, Cloxacillin, Gentamycin, Colistin, Carbenicillin, Kanamycin and Cephaloridine.

Hydrocephalus

This develops in over 80% of cases and in 100% of cases if the defect is in the thoraco-lumbar region. The commonest cause is the Arnold-Chiari malformation.

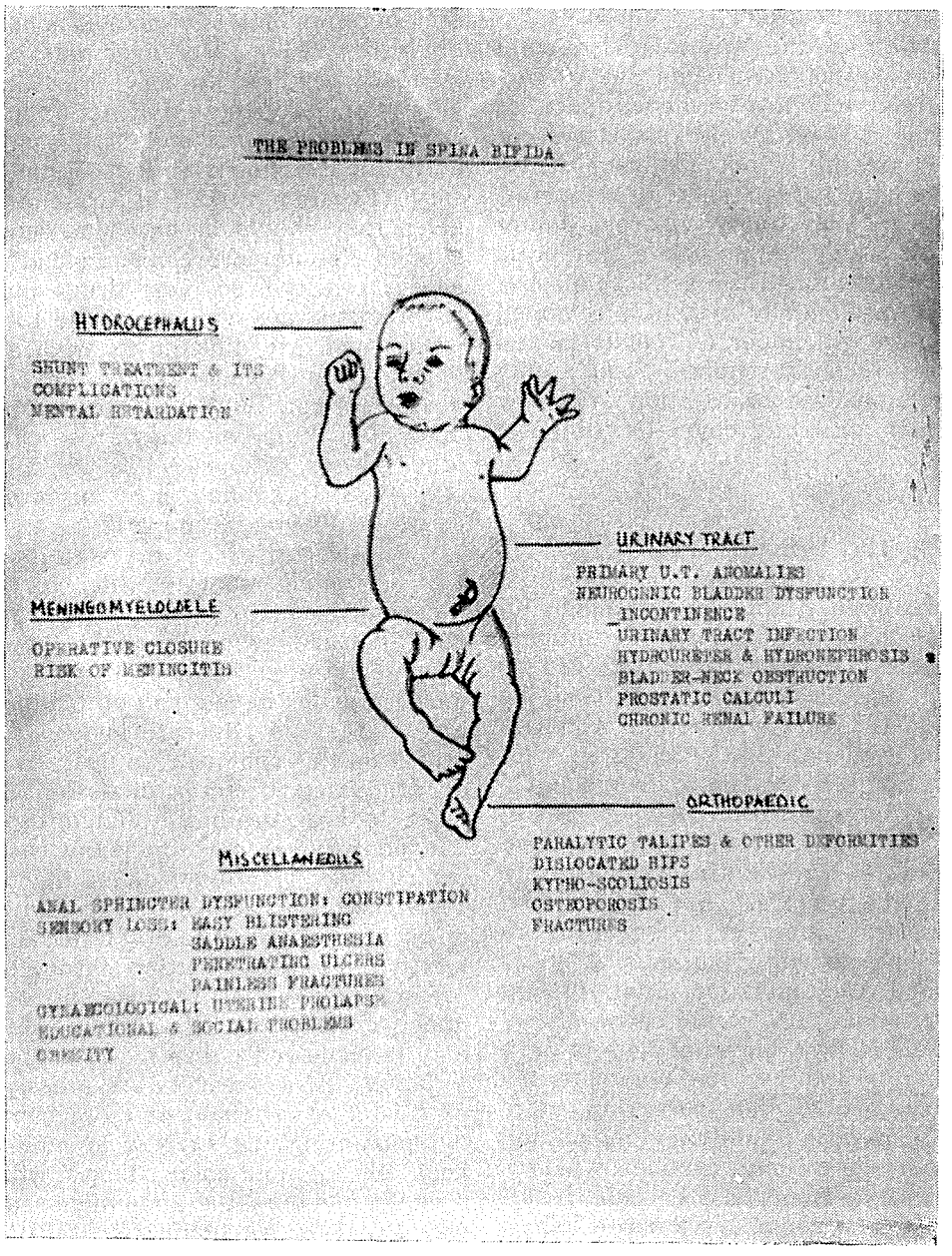
A great advance in treatment came in the early 1950s when the Spitz-Holter valve was introduced. This is a system of continuous C.S.F. drainage from the cerebral ventricles into the blood-stream via a one-way valve system. The Spitz-Holter valve is now used routinely by most paediatric surgeons, though neurosurgeons prefer modifications of it, like the Pudenz valve. Unless treatment is delayed too long, these methods allow the brain to develop normally and as a result the majority of these children, if adequately treated, are of normal intelligence. In Sheffield, routine air ventriculography is done in the first few weeks of life and this has shown that hydrocephalus is very often already present then — long before the head becomes clinically enlarged.

A series of controlled therapeutic trials carried out by Dr. Lorber and his group has given remarkably precise indications for operation. If on ventriculography the pressure is less than 300 mm water and the cerebral mantle is 25 mm or more thick, then no treatment is needed. These children will have a normal-sized head and grow up to be of normal intelligence. These make up about a third of all infants with hydrocephalus. Infants with extremely severe hydrocephalus and a cerebral mantle less than 15mm thick require early shunting preferably in the first week of life. These too have a good chance of normal mental development (Lorber 1968b). The largest number of infants lies between these two groups. Here, the decision is a difficult one but no operation is usually required if the head circumference is increasing at a rate of 10mm per week or less.

The initial operation for the insertion of a ventriculo-cardiac shunt is simple. Accurate placing of the catheter up to the level of D.4 under X-Ray control is used routinely by Zachary in Sheffield and Rickham in Liverpool, but others, notably Ellison Nash do not think this is necessary because the catheter will need elective revision later on in most cases owing to the child's rapid growth.

Meningitis is an absolute contraindication to the insertion of a shunt. In such cases a Rickham reservoir is extremely useful. This is a small plastic cap which is attached to the ventricular cannula at its point of connection with the upper end of the valve. Without needling brain substance and by needling the reservoir, C.S.F. under increased pressure can be removed and antibiotics can be injected daily into the ventricles till the infection is eradicated. Then, a distal atrial catheter is incorporated into the system.

While shunt insertion carries virtually no mortality, the complication rate is alarmingly high (Tsingoglu and Forrest 1971). Blockage of the shunt at the upper end due to either the choroid plexus or growing brain substance obliterating the holes in the silastic catheter is common.



This catheter may also pull out of the ventricular cavity or it may become detached from the proximal end of the Holter valve. In this serious situation the child will have symptoms and signs of increased intracranial pressure and the Holter pump will feel flat and empty as it cannot fill up from above. The upper catheter and its connection to the valve must be 'revised' as soon as possible. The shunt may also get blocked by organised blood clot at its lower end, either in the neck veins or in the right atrium. Occasionally, the lower end may get detached from the valve or at times may fracture. Symptoms are similar, but are slower in developing. The pump will feel stiff because one cannot empty it of its C.S.F. Treatment is not so

urgent here, but the lower catheter must be changed or 'revised'.

Infection of the shunt is also common, colonisation often being due to bacteria of relatively low virulence, such as *Staph.albus*. Low-grade pyrexia is present but blood culture is not always positive, though in some cases a spike of temperature or a rigor may follow vigorous pumping of the valve. Appropriate antibiotic treatment can control the septicaemia but unless the whole shunt system is removed the infection will linger on. Nicholas and others have recently reported good results with the immediate re-insertion of a new shunt under antibiotic cover (Nicholas *et al.* 1970).

The Urinary Tract

Spina bifida children have a high incidence of congenital urinary tract anomalies. These are however overshadowed by important complications arising from the neurogenic bladder dysfunction which is present in the majority of children with a lumbo-sacral defect. Poor detrursor muscle power, incoordination between detrusor and internal urethral sphincter and lack of normal bladder sensation leads to dribbling incontinence. There is also external urethral sphincter spasm with incomplete bladder emptying causing stasis and urinary tract infection. Scott (1970), found that about 50% of the cases assessed after their first birthday had urinary tract infection and 60% had complete urinary incontinence, this occurring more commonly in girls. Another complication is vescico-ureteric reflux leading to hydroureters and hydronephrosis. This is already present in about one-third of babies within a month of birth (Eckstein 1968). Hypertension and renal failure may later supervene.

The child's urinary tract should be investigated fully by means of repeated urine cultures (samples of urine are obtained by suprapubic bladder puncture), I.V. pyelograms, micturating cystic-urethrograms and, in difficult cases, cystometric pressure studies.

Prophylactic antibiotics will not reduce the incidence of infection and these are therefore not advisable (Zachary and Sharrard 1967). Moreover there is some evidence that the urinary tract infection may in many of these patients be localised to the bladder only and in this situation antibiotic treatment is indeed not necessary (Eckstein 1972). If antibiotics are given they should be based on sensitivity studies. The initial organism, usually *Esch. coli* is sensitive to most drugs but later re-infections are commonly due to organisms which are difficult to eradicate like Proteus and Pseudomonas. These often need treatment with parenteral injections of Gentamycin, Colistin, Carbenicillin and other newer antibiotics (Lorber and Formby 1968). Treatment must be prolonged, usually to beyond 3 months.

Surgical treatment plays an important part in management in order to prevent progressive renal damage. As the bladder-neck is usually wide open and obstruction occurs at the external urethral sphincter, most surgeons have given up bladder-neck Y-V plasty and advocate instead a direct attack on this sphincter: in girls, by means of graduated urethral dilatation and in boys, by transurethral sphincterotomy or pudendal neurectomy. In many cases, especially in girls, diversion of the urinary tract may be needed for social reasons or because of the risks of, or actual, deterioration of renal function through back-pressure effects or because of recurrent upper urinary tract infections. Diversion may be achieved either by cutaneous ureterostomy, if the ureters are grossly dilated (Lister *et al.* 1968) or more commonly by implanting the ureters in an isolated ileal or sigmoid-colon loop, which is brought out onto the abdominal wall surface (Cook *et al.* 1968). Generally speaking, urinary incontinence in boys can be well controlled by using a penile urinal bag. If however gross hydronephrosis is present, some diversion operation is required. Such treatment is also associated with many long-term complications. The use of continuous chemotherapy is debatable, but it is usually necessary to continue with it once it has been started (Scott 1970).

Orthopaedic Problems

The lower motor-neurone lesion in spina bifida produces flaccid paralysis of both legs. The factors which determine the extent and degree of muscle paralysis are: i. — the management of the spinal defect: early closure diminishing the degree of ultimate paralysis and ii. — the level of the lesion: the legs may be completely paraysed if the highest lumbar segments are involved. Unfortunately, the large majority of children with dorso-lumbar or lumbo-sacral defects are paralysed to a variable degree. Deformities and joint dislocations result from muscle action imbalance and no amount of isolated manipulation or plaster-casting can improve the situation without reconstructive orthopaedic surgery.

Paralytic dislocation of the hips is the biggest problem and occurs in about one-half of these children. A major advance in treatment has been the development of Sharrard's operation of posterior iliopsoas transplantation in which the still active psoas muscle is transplanted through a hole made in the wing of the ilium, fixing it to the greater trochanter of the femur (Sharrard 1971). This muscle acts as an abductor and hip stabiliser enabling the child to stand and later on to learn to mobilise himself. The operation is usually performed in the second year of life, but may be done much later. Other procedures like tenotomies, tendon transplants and osteotomies can deal with most deformities, especially those of the foot. This often means several major operations and the use of appliances supported by physiotherapy and education in walking. (Walker 1968). The use of retaining splints by irons, calipers and braces can be started in the second year of life, the object being to have the child standing at an age when the normal child does so. 'Skis' and parallel bars are necessary for walking instruction. The child is encouraged to be as mobile as possible and even a minor degree of ambulation adds greatly to the morale of the child and to that of his parents. The adoption of pessimistic attitudes in the early years will lead to complacent acceptance of wheel-chair life with all its drawbacks.

Educational Aspects

The intelligence range of these children without hydrocephalus is the same as that of the general child population and most of them will go to an ordinary school. The same can be said of about one-third of those with hydrocephalus, but the other two-thirds will require special schooling (Lorber 1970). In general, about 60% of children who have had treatment are of normal intelligence but because of their physical handicap many of these children have to attend special schools equipped with nursing and physiotherapy facilities. In the United Kingdom the Youth Employment Service is freely available to all school leavers and parents are encouraged to take full advantage of it. Many of these children will of course have to earn their living by mental rather than by manual work, but the more mobile child will usually have wider and better opportunities for employment.

Conclusion

In this article I have reviewed some of the most important aspects of the problems in the management of the child with spina bifida. As many specialities are involved it is most essential that treatment should be co-ordinated. Usually, liason between the specialities is undertaken by the paediatrician, but in some centres all aspects of the child's problem are dealt with in a "combined clinic" where the paediatrician, paediatric surgeon, orthopaedic surgeon, urologist, physiotherapists and medical social worker attend at the same time, At all times, the child's parents are supported by giving them information, advice, encouragement and help.

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NOTICE

This periodical is published biannually in June and in December. Contributions for the June issue are to reach the Editor at the Bacteriology Laboratory, St. Luke's Hospital, Malta, by the 1st April. They must be typewritten, with double spacing. References should be given by the author's name and by the year of publication. Papers, which are accepted on the understanding that they have not been published elsewhere, are to consist of reports of original work or studies or case histories.

We thank our advertisers for their valuable support.

MEDICAL NEWS

The following meetings of the Malta Branch of the British Medical Association were held:

On the 5th July, Dr. Joseph Muscat-Baron lectured on "Acute Coronary Care";

On the 6th July Professor Enno Freerksen of Hamburg spoke on "New experimental and clinical results in the field of leprosy treatment". This treatment depends on the use of Rifampicin, ethionamide and a long acting sulphonamide. Professor Freerkseen came to Malta at the invitation of the government and with the assistance of the Order of St. John; his treatment is being carried out, as a research project in our hospital and one looks forward with interest to the final result. It appears there have been some interesting findings so far.

On the 18th October, the Reverend Mgr. Professor Edward Coleiro gave the St. Luke's Day lecture, choosing as his subject "Some moral considerations on Transplants". The address was very interesting and produced an animated discussion.

On the 30th November Dr. Paul Cassar lectured on "The First Seventyfive years of Radiology in Malta".

The Malta Branch of the B.M.A., in co-operation with the Malta Branch of the Royal College of General Practitioners, held a very successful refresher course for general practitioners. Most of the St. Luke's consultants took part: Dr. J. Pullicino and Dr. J. Pisani, both of Mount Carmel Hospital, spoke on "Depression and its treatment by drugs" and on "Psychiatric Emergencies in General Practice" respectively. Mrs. Doreen Camilleri, a qualified physiotherapist, lectured on "Physiotherapy in general practice with special reference to stroke patients".

On the 11th December Professor T. H. J. Huisman of the Comprehensive Sickle Cell Centre of the Medical College of Georgia in the U.S.A., with whom Dr. J. L. Grech and Professor W. Bannister have been engaged in haemoglobin work, on a short visit to the island, lectured on "Foetal Haemoglobin and its abnormalities in health and disease".

We congratulate:

Dr. L. Spiteri on his being promoted to the Fellowship of the Royal College of Pathologists;

Professor Carmelo Coleiro on his being elected a member of the Faculty of Community Medicine of the Royal College of Medicine of the United Kingdom;

Dr. Alfred Portelli on his graduating as M.Sc in General Virology at the University of Birmingham last September;

Dr. G. Sant on his appointment as Radiologist at St. Luke's on the 2nd October;

Mr. Hector Galea on his appointment as Dental Surgeon and lecturer on Dental Surgery at St. Luke's on the 1st July;

Professor G. L. Howe of the University of London and Vice-Chairman of Council of the British Dental Association was in Malta in June serving as External Examiner. He lectured on "Surgical Aids to Orthodontics" to the Dental Association of Malta.

We chronicle with deep regret the death in August of Dr. Joseph Sammut, widely known in the profession for his work in the Radiology department; also the untimely death of Dr. Antoine Cremona, at the age of 46, on the 14th June; and the death of Dr. William Aquilina on the 26th November at 82. Dr. Aquilina had been Medical Superintendent of the Connaught and of the Central Hospitals.

The Associazione Italiana Centri Trasfusionali held its 18th Congress at the Hotel Verdala between the 31st October and the 5th November.

Welcome visitors to the homeland last summer were Dr. Cajetan Briffa from Victoria, Australia and Dr. Paul F. Bonnici from Los Angeles, California, besides several others.

Dr. Paul Cassar was last May elected a member of the Societè Internationale D'Histoire de la Medicine, which has its headquarters at Montpellier. On the 12th July he was elected to the Academie Internationale D'Histoire de la Pharmacie based at the Hague in Holland; no country has more than two full and two associate members at any one time. Dr. Cassar was also elected a Fellow of the Royal College of Psychiatrists, but, regretfully, he was

unable to accept the distinction for personal reasons.

In Dr. Cassar's paper on Dr. Victor Grima, which we published in the June issue, the word "son" at the 17th line in the second column on page 14 was inserted in error and should be deleted.

Dr. Daniel Micallef, at the Committee on Social and Health Questions of the Council of Europe, on the 7th July last, spoke on "The present state of General Practice in the Maltese Islands: an undeveloped essential community service".

The Ninth Congress of the International Academy of Legal and Social Medicine will be held between the 24th. and the 28th September 1973 in Rome. The secretariat is at the "Istituto di medicina legale" of the University of Rome at Viale Regina Elena 336, 00161, Rome.

PUBLICATIONS LIST

The following are recent publications by graduates of our Medical School:

AZZOPARDI, J.G. (with ZAYID, I.). 1972. Elastic tissue in tumpurs of salivary glands. J. Path., *107*, 149-156.

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FENECH, F.F. and GRECH, J.L. 1972. The incidence and pattern of poisoning in Malta. Jour. Eur. Toxic., No. 3, 208-212.

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GRECH, P. (With BEVIS, D.C.A. and PARSONS, R.J.) 1972. Radiation hazard in intra-uterine transfusion. Brit. J. Radiol., *531*, 193-196.

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GRECH, J.L. and BANNISTER, W.H. (With HUISMAN, T.H.J. and SCHROEDER, W.A.) 1972. Evidence for four nonallelic structural genes for the chain of human fetal hemoglobin. Biochemical Genetics, 7, 131-139.

PACE, J. LESLIE 1972 Atlas of the Cervical Spine. Abbottempo, 10(1), 26.

PACE, J. LESLIE 1972 Anatomy and the Art of Rembrandt — Scientia, 34, 149.

PACE, J. LESLIE 1972 The Anatomical features of prehistoric man in Malta. Published for Open Day Exhibition at the Royal University of Malta 19-20 May 1972.

PACE, J. LESLIE (with MARIE THERESE PODESTA') Some *in vitro* observations on the pharmacological responses of human and guinea-pig taenia coli.

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