Conclusions

In the comparatively few centres in this country where the cytological diagnosis of cancer has been practised for some years the successes have been such that the demand for its use has tended to increase. This is exemplified at the Royal Free Hospital, where during a brief period of some six years the successes of one or two research workers have resulted in the establishment of a department devoted exclusively to cytological cancer diagnosis with facilities for both teaching and research work. During this time the great advantages of the technique have become apparent. Firstly, and most important, as a routine procedure it can be used to detect tumours at a very early stage of development, often before signs and symptoms appear. It is simple to perform and need cause no alarm, inconvenience, or discomfort to the patient, and thus it can be repeated as often as may be necessary. The only inconvenience that a patient may be caused is in obtaining those materials where needles or other instruments have been used.

The chief disadvantages of the method are the time that is required to read the slides and the lack of trained personnel to do this. The taking and processing of the smears is simple, but the interpretation of the wide range of the cellular variations in the different types of body fluids requires considerable training. Until recently it was usually found necessary to go to America to gain the requisite experience, but now one or two cytological centres in London and the provinces provide facilities for teaching the technique and the interpretation of the smears. This should help to eliminate the necessity for sending personnel abroad for training, with the concomitant expense which that involves.

A plea is made for the more extended use of the method throughout the British Isles, where much of the original pioneer cytological work was done. The experience of cytological workers all over the world shows that the technique is well worth while, for if the detection of cancer when it is symptomless and at a very early stage of development is going to save or prolong lives—and there is reason to believe that it does—then the method is a most valuable weapon in the fight against cancer.

Summary

An account is given of the cytological technique for the early detection of malignancy which, though evolved in the early days to a great extent by British pathologists, was developed by Papanicolaou in America in such a way that it has practical application.

The principal and most successful uses of the method are described and reference is made to the processing, staining, and interpretation of the smears.

Although most of the published work on cytology comes from American and Continental laboratories, the references in this paper apply chiefly to publications from British cytologists.

A short account is given of the establishment of a cytology department and its use at the Royal Free Hospital during the last two years, with a record of the results obtained. Typical example cases are quoted. The advantages and disadvantages are discussed, and a plea is made for the more extended use in this country of this undoubtedly valuable weapon in the fight against cancer.

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THYROGLOBULIN ANTIBODIES IN 1,297 PATIENTS WITHOUT THYROID DISEASE

BY

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Since the work of Roitt and Doniach (1960) and others it is known that the sera of patients suffering from thyroid disorders, especially Hashimoto's disease, often show antibodies to thyroglobulin. The present investigation reports the frequency with which antibodies may be found in subjects not suffering from any apparent thyroid abnormality.

Materials and Methods

The sources of the sera tested were 597 samples sent to the laboratory for performance of the Wassermann test and 700 sent to the blood transfusion service for grouping, the former preserved at 4° C. for a week and the latter at -20° C. The notes were examined of all patients whose sera contained thyroid antibodies, and cases with clinically observed thyroid abnormalities were removed from the main series and made into a subgroup for the purpose of comparing the incidence of complement-fixing antibodies in the two groups (see Table II).

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The sera were estimated by the tanned red cell (T.R.C.) haemagglutination test, using formalized sheep cells coated with purified thyroglobulin, prepared by Dr. A. J. Fulthorpe (Burroughs Wellcome). A screening procedure was developed in order to select sera for full titration, using a platinum loop which could be flamed rapidly between each specimen. A loop of 6 mm. diameter (as used in bacteriology) when dipped into serum was found to pick up a minimum quantity of 0.0025 ml.

0.03 ml. (one drop from a standard pipette) of thyroglobulin T.R.C.s (1:100 solution) and 0.03 ml. of normal saline were added to each well of a "perspex" tray. The platinum loop was flamed to white heat, allowed to cool, dipped into the test serum, inspected to see that a film had formed across the loop, then agitated in the measured volume of tanned cells until the film broke. The wire was again flamed, and the procedure could be repeated with further test sera.

This screening method gives a minimum dilution corresponding to a 1:10 conventional titre (1:20 actual dilution). It was thought that, although many 1:5 titres would be missed by this procedure, all titres of 1:25 and above should be detected. Ten sera previously established as positive at 1:25 and 10 positive at 1:250 were screened by the above method, and all were found positive.

Owing to the detection of titres of less than 1:5 (the loop could pick up two to three times the minimum) and the presence of heterophil sheep-cell antibodies the screening method produced many false positives, but this did not affect the final result, as all sera found positive were submitted to a full titration (Roitt and Doniach, 1958), preceded by absorption with uncoated formalized control sheep cells. All positive sera (including those from patients with clinically observed thyroid abnormalities) were also examined for complement-fixing antibodies using extracts of thyrotoxic thyroid and other organs. The antigenic activity in the saline extract of thyrotoxic gland is due chiefly

TABLE I.—*Tanned Red-cell (T.R.C.) Agglutination—Results by Sex and Age-group*

Age-group*	Female				Male			
	Total Tested	1:5	1:25	1:250	Total Tested	1:5	1:25	1:250
0-19	47	1	0	0	50	0	0	0
20-29	182	1	5	1	120	1	1	0
30-39	132	2	3	1	79	1	0	0
40-44	49	1	2	2	75	3	1	2
45-49	60	1	1	5				
50-54	62	3	3	2	134	1	0	1
55-59	50	4	5	1				
60-69	83	4	7	1	85	2	1	0
70+	41	2	0	0	48	1	1	0
Total	706	19	26	13	591	9	4	3

* Positive sera of patients with recognized thyroid abnormalities have been excluded.

TABLE II.—*Proportion of T.R.C. Agglutination Positive Sera at Each Titre which Contain Antibody to Extracts of Thyrotoxic Gland (C.F.T. +)*

T.R.C. Titre	Non-thyroid Disease		Known Thyroid Abnormality	
	Total in Each Group	C.F.T. +	Total in Each Group	C.F.T. +
1:5	27	4+2*	3	2
1:25	29	3	3	0
1:250	15	1	6	5
1:2,500	1	0	3	2
1:25,000	1		2	1
Total	73	8+2	17	10

* These two patients showed complement-fixing antibodies to organs other than the thyroid—for diagnosis, see text.

to the microsomal fraction. The other antigens were simple saline extracts of each organ. Details of the complement-fixation technique were as described in Roitt and Doniach (1958)—briefly, 2 M.H.D. of complement incubated for one hour with the antigen and test serum, then read after 30 minutes' further incubation with the indicator cells.

Results

1,297 sera were screened and titrated as described. The results, grouped according to sex and age, are shown in Table I and the Charts. 17 positive sera from patients with observed thyroid disease have been excluded from Table I. They are compared with the "normal" group in Table II. As already mentioned, many sera containing a 1:5 titre will have been missed by this method, but as there is no reason for thinking that the proportion missed varies between the groups the positive results at this dilution are included. Although the data at 1:5 have no absolute value, they can still be used for comparing groups. It can be seen that positive sera at 1:25 and above are six times more common among women than among men. At ages 40 to 69, 10% of all women in this series have antibodies to a titre of 1:25, 1:250, and in one case 1:2,500. At 70 and over there are no positives greater than 1:5 among the women. In the 20-39 age-group, 8 (5%) positive sera were found among the 174 non-pregnant and 2 (1.5%) from the 140 pregnant females ($\chi^2=2.7$. $P=0.10$).

The number of positive male sera is too small for analysis, but a peak in the 40-49 age-group is suggested, corresponding to the more obvious one in the females.

Complement-fixing antibodies to thyroid at a minimum titre of 1:4 were demonstrated in 10 out of 17 sera from patients with known thyroid abnormalities, but in only 10 of the 72 subjects with apparently normal thyroids. Two of these latter 10 gave positive reactions with all other organs, and thus probably contained not the thyroid microsomal antibody but the non-specific anticytoplasmic antibody (Gadjusek, 1958). Of the group derived from the W.R. laboratory, one (a woman aged 54) with a positive T.R.C. agglutination (titre 1:5) was also W.R. positive.

Discussion

Hackett, Beech, and Forbes (1960b) gave published figures for the incidence of T.R.C. antibody titres of 1:10 and above in a group of 387 mixed medical patients at Adelaide. They found that 25.7% of women aged 30 and over were positive at this titre. Unfortunately their series is not comparable with the one here presented, as the discriminating titre selected is different, the sensitivity of their tanned-cell system is probably greater than here, and 15 of their 68 positives were suffering from "collagen" and allied diseases in which there is a generalized increase in antibody activity. The chief qualitative differences are that in the present series of women a sudden increase in the number of positive sera is found in the fifth decade as against the fourth decade in Adelaide, where they persist into oldest age as against the suggestion of a falling off in the over 70s in this series. (Comparison of the female over 70s with the 40-69 age-group gives $\chi^2=3.38$, $0.05<P<0.1$.) The sex ratio of positives, corrected for the unequal numbers of the sexes in the populations examined, in Adelaide is 1:2.5 male to female as against 1:4.5 here. (Comparison of actual numbers involved gives $\chi^2=4.65$, $P<0.05$.) If only the 1:5 titres are taken into account

the ratio becomes 1:1.75—much closer to the Adelaide figure—and consequently the difference between the two series is probably due to the weaker titres accepted by Hackett *et al.*, at which lower levels the relation between sex and antibody production may be less marked.

It is interesting that the sex ratio of 1:4.5 compares closely with the 1:4 male/female ratio commonly given for clinical myxoedema (Means, 1948). Fig. 3 shows the distribution of age of onset by decades of 107 females suffering from primary myxoedema and/or Hashimoto's disease seen in recent years at the Middlesex Hospital. It can be seen that there is a resemblance between the age distribution of these patients and those of the present series, with a similar peak in the fifth decade.

In another series, Blizzard *et al.* (1960) found agglutinating antibodies in 1.8% pregnant women as against 3% in the general population; but their general group was not a matched population, as it included male and female subjects 17 to 70 years of age.

In the present series, owing to the source of the sera, there are many pregnant women, males healthy apart from possible venereal disease, surgical admissions (routinely blood-grouped), and patients from a large radiotherapy unit. Owing to this bias no special significance can be attached to the diagnoses given in Table III. For example, the apparent prominence of

TABLE III.—*Diagnoses of Patients whose Sera Contained a Titre of T.R.C. Agglutinating Antibodies 1:250 and Above*

Intermenstrual bleeding (due to proliferative endometrium)
Fibroids
Broad-ligament cyst
Haemorrhage into ovarian follicle
Squamous metaplasia of cervix
Carcinomatosis (breast)
Hodgkin's disease
Chronic lymphatic leukaemia with multiple transfusions (1:25,000)
Varicose veins
Hernia
Gall-stones and carcinoma of uterus (1:2,500)
Osteoarthritis of hip
Gastric ulcer
Epilepsy

gynaecological conditions arises because the main source of sera in the middle-aged group, in whom antibodies seem especially common, is almost exclusively from women admitted for gynaecological operations. It has been suggested that there is a relation between disorders of the thyroid and disease of the female genital tract, but this contention is far from proved.

The more common finding of complement-fixing antibodies in patients with thyroid disease than among

"normals," despite their both showing agglutinating antibodies, accords with the experience of Roitt and Doniach (1958), who demonstrated that, of those sera containing agglutinating antibodies, complement-fixing antibodies were present in practically all cases of Hashimoto's disease, in two-thirds of spontaneous adult myxoedema cases, in about one-half of thyrotoxic, and in one-seventh of patients with non-toxic colloid goitre. Goudie, Anderson, and Gray (1959) have studied the incidence of complement-fixation in a hospital population not suffering from overt thyroid disease. They found a sex ratio of 1:5 male to female, but the peak frequencies were clearly in the sixth and seventh decades. They were able to examine a number of the thyroids of these patients after death, and found a high correlation between C.F.T. positive sera and areas of lymphocytic infiltration. Their work also correlates well with Simmonds's (1913) observations on lymphocytic islands in the thyroid, as he also found a very definite peak in the sixth and seventh decades, falling off thereafter.

Unlike Hackett, Beech, and Forbes (1960a), who found that 8% of healthy blood donors had positive complement-fixation tests to at least one of a selection of organs, of the 90 sera tested in the present series (all those containing agglutinating antibodies), only two gave complement-fixing antibodies against organs other than the thyroid. One patient had a carcinoma of the cervix with bilateral ureteric obstruction and had been treated by radiotherapy, the other was a pregnant woman aged 29 with a history of recurrent acute arthropathy over the preceding three years. Hackett's method employs a smaller amount of complement, with a consequent greater sensitivity than the method used here. Their thyroid antigen was prepared from normal thyroid tissue and accordingly their results are not comparable with those of the present series, in which the antigen was extracted from thyrotoxic gland.

Summary and Conclusions

It has been established that thyroglobulin antibodies are commonly found in patients with apparently normal thyroids. The age-sex distribution found in subjects with antibodies is very similar to the age-and-sex pattern in patients with myxoedema and Hashimoto's disease, with a maximum in the fifth decade, but both preceded by 10–20 years the peak frequency of lymphocytic infiltration and associated positive complement fixation.

FIG. 1

FIG. 2

FIG. 3

FIG. 1.—Distribution in decades of women with positive sera 1:25 and above. (Constructed from data in Table I.) FIG. 2.—Comparison in three age-groups between men and women with positive sera 1:25 and above. (Constructed from data in Table I.) FIG. 3.—Distribution of estimated age at onset of 107 patients suffering from hypothyroidism and/or lymphadenoid goitre seen in recent years at the Middlesex Hospital.

The figures reported here show that over 10% of middle-aged women have circulating thyroid antibodies.

Some of these women probably proceed to overt thyroid deficiency with high titres of antibodies, but the factors which influence progression are as yet unknown.

Theoretical considerations apart, it is hoped that the data here presented will provide an empirical basis for estimating the significance of antibody levels in the diagnosis of thyroid disease.

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PHENFORMIN AS ADJUVANT ORAL THERAPY IN DIABETES

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The sulphonylurea group of drugs were the first to be used successfully in the oral treatment of diabetes (Franke and Fuchs, 1955; Bertram *et al.*, 1955). It soon became apparent that these drugs were ineffective in severe diabetics with a tendency to ketosis, and their use is now restricted to milder diabetics who cannot be controlled by simple dietary restriction. Two drugs of this type are in common use—tolbutamide and chlorpropamide. The latter is the more powerful hypoglycaemic agent and has a more prolonged action (Granville-Grossman *et al.*, 1959). However, not all diabetics who might be expected to respond to chlorpropamide do so in practice. Some newly diagnosed diabetics whose urine is free from ketones fail to respond satisfactorily to chlorpropamide, while others who have responded initially begin to relapse into hyperglycaemia gradually after a time.

Phenformin is a diguanide, and earlier trials showed that, although it is a most potent hypoglycaemic agent and sometimes successful where chlorpropamide has failed, it is poorly tolerated when administered at its maximum therapeutic level, often giving rise to gastro-intestinal disturbances (Hall *et al.*, 1958). Its mode of action is uncertain but differs from that of the sulphonylureas (Marble, 1960). Hence, in patients who show an inadequate response to the sulphonylureas, phenformin

has been used as an addition to these drugs (Beaser, 1958, 1960; Granville-Grossman *et al.*, 1959; Unger *et al.*, 1960). Given in this way, the dose of phenformin can be reduced sufficiently to avoid gastro-intestinal intolerance.

The purpose of this communication is briefly to review the present overall picture of tablet therapy in a diabetic unit, and, in particular, to define the role of phenformin as adjuvant therapy to the sulphonyl ureas.

Overall Picture

Suitable records were available for 1,450 diabetic patients treated at Whittington Hospital Diabetic Clinic. These were divided into three groups: those controlled by simple dietary restriction alone, those taking tablets, and those requiring insulin. These groups are not static. Many diabetics needing insulin or tablets for initial control later remitted and were managed on simple dietary control (Bloom, 1959). Others who were well controlled for many years on dietary restriction later began to relapse and needed tablets or insulin. Some who were well controlled initially by tablets later relapsed and needed insulin.

The large majority of young diabetics required insulin, and only a few could be controlled on diet or tablets (Table I). In those over 50, however, more than 20%

TABLE I.—Treatment of 1,450 Diabetics According to Age

Age:	0-30	31-50	51-70	71+
Insulin ..	111 (87.4%)	150 (52.6%)	210 (31.4%)	137 (37.2%)
Diet alone ..	12 (9.5%)	104 (36.5%)	320 (47.7%)	151 (41.0%)
Tablets ..	4 (3.1%)	31 (10.9%)	140 (20.9%)	80 (21.8%)
Total ..	127 (100%)	285 (100%)	670 (100%)	368 (100%)

were controlled by oral therapy. The majority of patients controlled by tablets were taking either chlorpropamide or tolbutamide (Table II). In recent years

TABLE II.—1,450 Diabetics According to Treatment

Insulin ..	608
Diet alone ..	587
Tablets ..	255
Chlorpropamide ..	107
Tolbutamide ..	94
Phenformin ..	14
Chlorpropamide plus phenformin ..	36
Tolbutamide plus phenformin ..	4

chlorpropamide has been used in preference to tolbutamide in new cases, but patients already satisfactorily controlled by tolbutamide have continued on this therapy. 40 patients, representing 16% of those on oral therapy, or 2.7% of all diabetics, required phenformin in addition to either chlorpropamide or tolbutamide.

Phenformin as Adjuvant Therapy.—Phenformin was used as adjuvant therapy to the sulphonylureas in diabetics showing primary or secondary failure of response to tolbutamide or chlorpropamide. Primary failure was noted in diabetics not responding to diet, and also in diabetics previously taking insulin. Secondary failure occurred in patients who were controlled by tolbutamide or chlorpropamide for at least a month but who then began to relapse on this therapy despite adherence to diet.

Method of Trial

Diabetics Not Responding to Diet and Showing Primary Failure to Respond to Tolbutamide or Chlorpropamide

New cases not severe enough to need insulin were placed on a restricted diet varying from 800 calories a day (80 g. carbohydrate) in very obese patients to 1,500 calories (175 g. carbohydrate) in those of normal weight,