Though the infant appeared well after the attack of acute viral hepatitis, there was persistent hepatosplenomegaly with raised transaminase and bilirubin levels, indicating active hepatitis during the progression to cirrhosis associated with giant cells. Aterman (1963) reported two siblings who died with giant cell hepatitis after an attack of infective hepatitis in the mother, and strongly favoured the view that giant cell hepatitis of the newborn is a form of viral hepatitis acquired from the mother. The detection of the Au antigen in the mother and infant here described provides evidence to support this view, and since the mother has become a chronic carrier her future children will presumably be at high risk of developing neonatal hepatitis.

It is not known whether the Au antigen is the virus itself, a viral protein coat, or a product of liver cell damage, but it is of interest that the large (40–45 nm.) particles thought by Dane *et al.* (1970) to be the parent virus were present throughout in the infant's serum. The significance of the large particles with surface projections resembling paramyxoviruses which we have detected in recent specimens of the infant's serum is also uncertain. We have observed similar particles in other specimens of serum from patients with acute and chronic hepatitis, including both Au-antigen-positive and Au-antigen-negative sera. They bear some resemblance to the coronavirus-like particles described by Zuckerman *et al.* (1970) in the serum of a patient with chronic active hepatitis.

The development of antibody to the Au antigen in the infant as detected by electron microscopy and by immunodiffusion and the rise in IgG levels is of interest in view of the suggestion of Almeida and Waterson (1969) that antibody may be responsible for tissue damage in hepatitis.

The mother's occupation as a dental assistant may have greatly increased her risk of becoming infected with the long-incubation-period serum hepatitis virus, and the laceration of the infant's scalp may have facilitated its transmission, so that the circumstances under which the infant contracted Au-antigen-positive chronic active hepatitis are exceptional.

Though such exceptional circumstances may be necessary in this country, the Au antigen may prove to be an important cause of cirrhosis in communities where the carrier state often occurs.

As with most other cases of Au-antigen-positive chronic active hepatitis the smooth muscle antibody was not present in this infant's serum though it is usually detected at high titre in the serum of patients with Au-antigen-negative chronic active hepatitis, thus raising the possibility that these two groups are aetiologically distinct (Sherlock *et al.*, 1970; Vischer, 1970; Wright, 1970).

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ADDENDUM: Since this report was submitted for publication C. Sirtori, *British Medical Journal*, 1970, 3, 768, has detected structures resembling paramyxoviruses by electron microscopy of liver biopsies from children with acute viral hepatitis which appear to be identical to one of those illustrated in Fig. 3 which we have observed in this infant's serum.

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Preliminary Communications

Bioassay of Thyroid Stimulating Hormone Using the Goldfish

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Summary: A new method for the bioassay of thyroid stimulating hormone using goldfish is described. The technique can detect thyroid stimulating substances in euthyroid unconcentrated plasma. Early results show that the method compares well with other techniques. Some evidence has been obtained which suggests that in primary myxoedema there may be some impairment of the thyropituitary "servo-mechanism" in older patients.

INTRODUCTION

Many methods for the bioassay of thyroid stimulating hormone (T.S.H.) have been developed (Brown, 1957), but there has been considerable difficulty in finding a simple technique with the necessary sensitivity to detect thyroid stimulating activity in normal plasma. Others have shown that fish can be used as subjects for the bioassay of T.S.H. (Fontaine and Fontaine, 1956; Brown, 1957); and it has also been shown that fish are very sensitive to other thyroid stimulating substances such as heterothyrotrophic factor. The available evidence (Fontaine and Burzawa-Gérard, 1966; Fontaine, 1969) suggests that heterothyrotrophic factor is indistinguishable in its activity from mammalian follicle stimulating hormone (F.S.H.). Nevertheless, the term heterothyrotrophic factor has

been retained because it remains to be shown that this holds true in the bioassay system described here.

Though immunoassay has become popular as a means of assaying T.S.H. the method has some limitations. The antigen used to raise the specific antibody (human T.S.H.) is still an impure substance (Bates and Condliffe, 1966) and its very specificity excludes the study of other physiologically active substances in human plasma such as long-acting thyroid stimulator (L.A.T.S.) (Adams and Purves, 1956) and possibly heterothyrotrophic factor (Fontaine and Lopez, 1965). It also excludes the discovery of any further such substances that may be present. While T.S.H. extracts remain impure it will be necessary to have effective bioassay procedures for reference.

A brief description of the method adopted, together with some preliminary results, is given here. Initially we thought that hypophysectomy would be needed to achieve sufficient sensitivity in the goldfish, and this was tried, but further experiments showed that heated intact fish gave results which were even more sensitive and precise.

METHOD

Goldfish of the comet variety, 2–3 in. (5–7.5 cm.) long, are kept in compartments in a 100-litre tank. All compartments are interconnected in a common environment with central filtration and heating and a pumped circulation of water. The water temperature is gradually increased over two days until

it reaches 32°C., and is subsequently maintained at this level; the fish are ready as soon as this temperature is reached.

In each assay "run" a dose response curve is obtained for standard bovine T.S.H. Three dilutions are used, made up in the plasma of outdated human bank blood heated to 60-65°C. for three hours, so that the total volume injected per fish contains 10 or 100 or 1,000 μ units of bovine T.S.H. Groups of six fish are used at each strength, and six fish are also used for each of the unknown plasma samples. Radioactive iodine (high specific activity Na^{131}I in 0.9% saline) is added to standards and to unknown plasmas to give a final dilution of about 3 $\mu\text{Ci}/\text{ml}$. All fish are injected with a volume of 0.3 ml., given as one intraperitoneal injection of 0.15 ml., and as intramuscular injections into both flanks of 0.075 ml. A 30 gauge lymphangiogram needle is used.

The fish are rinsed immediately after injection and then transferred individually to 150 ml. of clean water in a plastic container. Their radioactive content is then assayed in a large volume 4 π plastic scintillation counter (Smith, Clarke, and Reed, 1967). Radioactivity is again measured (after rinsing each fish each time) at 6 and 72 hours after injection. Comparison of immediate post-injection and six-hour activity ensures the detection of a "leaking" fish, which is rejected. Because each fish initially receives about 1 μCi of ^{131}I adequate statistical accuracy can be obtained with short counting times.

Response is measured in terms of the retention of ^{131}I with time after injection, as the ratio of the activity at 72 hours to that at six hours, expressed as a percentage. There is a linear relationship of this response to the log dose (Fig. 1). Values for the unknown plasmas are then read off the line so constructed (Fig. 1).

FIG. 1—A dose response plot indicating how three unknowns (Nos 1-3) may be read off the line so constructed. The response is measured as the three-day counts/six-hour counts expressed as a percentage (see text). Six fish are used for each dose of bovine T.S.H., and each unknown and the vertical bars indicate the scatter within each group. λ = Standard error of the estimate/slope. P value is for the significance of the linear regression against log dose.

RESULTS

Sensitivity.—Linearity of response against log dose can be maintained down to amounts of 8-10 μ units of bovine T.S.H. per injected volume of 0.3 ml.; thus the lowest detectable level of T.S.H.-like activity is about 33 μ units/ml.

Precision λ (as indicated by the standard error of the estimate/slope).—This varies between 0.28 and 0.64, with a mean value for the last six experiments of 0.435.

Standards.—Samples of human T.S.H. (Research Standard A) obtained from the Medical Research Council were estimated at a potency of 51 μ units per ampoule (fiducial limits 31-80 μ units/amp). Other estimates of this preparation by bioassay vary from 4 to 108 μ units/amp. (National Institute for Medical Research, Mill Hill). The slopes of response to bovine T.S.H. and the human T.S.H. remained very nearly parallel over the range of values as follows:

Linear Regression of Response Against Log Dose						Range	Slope
Human T.S.H.	..	..	..	..	..	0.0198—1.98 μg .	16.3
Bovine T.S.H.	..	..	..	..	..	27—2,700 μ units	16.1

CLINICAL RESULTS

Results are shown from the first 21 patients to be studied with this technique (Fig. 2). With these and more recent data the levels in primary myxoedema (12 patients) range from 198 to 1,100 μ units/ml. (mean 441 μ units/ml., S.D. 302), and in normal individuals (nine patients) from 33 to 160 μ units/ml. (mean 87.5 μ units/ml., S.D. 43.6).

FIG. 2—Results in clinical categories: T.S.H. results are compared with T_3 and P.B.I. results in each patient.

There had been some difficulty in the past in correlating the results of T.S.H. assay with other measurements of thyroid function. It is, however, reasonable to assume that a log hormone concentration to target organ response relationship will exist in the intact system as well as in a bioassay preparation. In Fig. 3 the T.S.H. concentration in μ units/ml. is plotted on a logarithmic scale against the T_3 resin uptake value (Thyopac). For all the 21 subjects the correlation coefficient for the linear regression was 0.845. If, however, only the younger group of 13 subjects is included it is highly significant at 0.947. It can be seen that in the myxoedema range the values achieved by the older subjects (≥ 50 years) give a wider scatter to the data by being universally lower for a given T_3 estimation than those of the younger subjects. It must be acknowledged that the data are at

FIG. 3—T.S.H. concentration in μ units/ml. plotted on a logarithmic scale against T_3 resin uptake value. Patients with primary myxoedema are considered together with some normals but are split into two age groups. ○ = Patients aged 50 and over. ● = Patients under 50.

present scanty, but it would seem that the T.S.H. response of older patients with primary myxoedema is reduced.

DISCUSSION

Details of the method have not been given here but will form the subject of a later report. This bioassay technique is sensitive enough to detect thyroid stimulating substances in euthyroid human plasma without the need for preliminary concentration of the plasma activity. This degree of sensitivity is higher than that generally achieved by other bioassay methods, and it is possible that substances other than T.S.H.—for example, heterothyrotrophic factor—may be detected, and may therefore contribute to the activity levels measured. The

activity nevertheless seems likely to be of pituitary origin, because in hypophysectomized and hypopituitary patients none is present. The range for euthyroid subjects so far (33–160 μ units/ml.) is similar to that of other bioassay methods (0–160 μ units/ml.) (Raud and Odell, 1969) when bovine T.S.H. is used as the standard.

Certainly the activity levels detected by this method in thyrotoxicosis are considerably different from those found when immunoassay methods are used. This difference may be due to L.A.T.S.-like substances or to heterothyrotrophic factor. Further work is in progress in order to define the part played by these and other possible components.

The wide range of T.S.H. values found in primary myxoedema (also noted by others) appears from the limited data at present available to be explicable on the basis of a diminished responsiveness of the pituitary in the older patient.

Practical features of this method are that the fish are inexpensive and that stocks are easily maintained. No special facilities are required, and the simple tank system required takes up only a small space. Equipment (circulation pumps, water heaters, aeration pumps) is readily available at small cost.

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Medical Memoranda

Extensive Keratoderma Blennorrhagica in a Woman with Reiter's Disease

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Reiter's disease is unusual in females, the sex incidence of this condition being reported as 15 males to one female (King and Nicol, 1969). Keratoderma blennorrhagica is the name given to the skin manifestation of this disease, and the skin of the soles of the feet is the commonest site involved. It is found in about 8% of patients who develop the venereal type of Reiter's disease, and in only 1% of those with the dysenteric type (Hancock, 1965).

A case of severe Reiter's disease in a young woman who developed extensive keratoderma blennorrhagica is described.

CASE REPORT

A 20-year-old woman was admitted to hospital with a two-week history of fever (not responding to salicylates) and pain in the right ankle, left hip, and lower part of the back. She had noticed a yellow vaginal discharge for three days but denied ever having had intercourse. There had been no disturbance of bowel habit and no previous significant illness.

On examination she was pyrexial (101°F.; 38.3°C.), pale, and obviously unwell. There was swelling and limitation of movement of the right ankle, and movement at the left hip joint was painful but not restricted. A red area resembling an incipient boil was seen over the left shin. There was no lymphadenopathy, and no other abnormality was found on examination. Haemoglobin was 11.6 g./100 ml.; W.B.C. 7,100/mm.³; and E.S.R. (Wintrobe) 9 mm. in the first hour. Radiographs of chest, ankles, pelvis, hip joints, and sacroiliac joints were normal. Urine culture grew *Escherichia coli*, which was treated with ampicillin, but she remained ill and pyrexial. Blood cultures were negative.

After five days in hospital she developed a vesicular eruption on the left heel and under the left hallux, typical of keratoderma blennorrhagica. By this time the arthritis had spread to involve the right elbow, left knee, and the metatarsophalangeal joints of both feet. Rose-Waaler and latex-rheumatoid antibody tests were negative, and L.E. cells were not found. She developed cervicitis and urethritis but not conjunctivitis. Vaginal smears showed *Candida albicans* on several occasions, and several groups of Gram-negative intracellular diplococci were seen on one occasion. An attempt to culture the gonococcus failed, as the culture medium had become overgrown with *Proteus* species. Healing erosions were seen on the tongue, and painless superficial erosions were present on the hard palate. Blood Wassermann and Kahn tests were negative.

The gonorrhoea was treated with a course of intramuscular penicillin but without improvement in her general condition. The