ample published evidence that severe liver disease can lead to haemodynamic changes, and the occasional occurrence of cardiac failure is to be expected.

Sources of error are likely to arise from attributing the cardiac failure to cor pulmonale, especially in men, to valvular disease, or to congenital heart disease in children. Constrictive pericarditis may also be considered in view of the gross oedema, but the quiet cardiac impulse and the low output state should serve to distinguish it. Jaundice may be slight or absent, and if present is attributed to venous congestion. Other evidence of liver disease is masked by the anasarca. A careful search may reveal signs of liver failure such as fetor hepaticus and a coarse tremor, though the latter is by no means specific and may occur in other forms of cardiac failure (Conn, 1958). It is debatable whether liver failure ever results from "cardiac cirrhosis" (Sherlock, 1951), though if it does it is likely to be the end-result of chronic rheumatic heart disease and therefore not a source of confusion.

Treatment in the above cases was unsatisfactory. The standard regime of bed rest, salt restriction, and diuretics had little influence on the oedema. It is possible that attempts to improve the body proteins (provided there is no clinical evidence of liver failure) might prove successful, or that other methods of promoting diuresis, such as the use of steroids or spironolactones and osmotic diuretics (Shaldon *et al.*, 1960), would be justified. This presupposes a correct diagnosis of the cause of the cardiac failure. One fact that emerges is the disastrous effect of removal of oedema fluid by mechanical means. It is well known that abdominal paracentesis is poorly tolerated by patients with portal cirrhosis and is best avoided; the same obviously applies to the use of Southey tubes. The reason almost certainly is that these procedures cause marked alterations in electrolyte and water distribution. There is, however, need for a detailed study of their effect in cirrhotics, similar to that of Vere and King (1960) in patients with the nephrotic syndrome.

Summary

Three cases of cardiac failure believed to be secondary to portal cirrhosis are described.

Treatment was unsuccessful and the removal of oedema fluid appeared to hasten death.

Prompt recognition of the cause of the cardiac failure might lead to more effective therapeutic measures.

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Preliminary Communications

Biological Method for Detection of Parathyroid Hormone

Previous methods for the biological detection of parathyroid hormone depend on changes in serum calcium values in the intact animal (Collip and Clark, 1925; Bliss and Rose, 1940; Biering, 1950), in the parathyroidectomized animal after injection of parathyroid extract (Davies, Gordon, and Mussett, 1954; Munson, 1955), or on the determination of urinary phosphate in normal or parathyroidectomized rats after administration of parathyroid hormone (Tepperman, L'Heureux, and Wilhelmi, 1947; Davies and Gordon, 1953; Kenny, Vine, and Munson, 1954; Davies, Gordon, Mussett, 1955); the smallest amount of hormone detectable by these methods is 2.5 U.S.P. units (Davies *et al.*, 1955). A technique for the detection of smaller quantities of parathyroid hormone is presented in this paper. It is based on increase in serum alkaline phosphatase activity after "parathormone" injections into parathyroidectomized rats. No such increase occurs in non-parathyroidectomized rats.

METHODS

Two-weeks-old female rats of the hooded Norwegian variety, maintained before and after operation on a standard cube diet (No. 41, Bruce and Parkes, 1949) and weighing approximately 20 g., were used in all the experiments.

Parathyroidectomy.—The rats were anaesthetized with ether and a midline incision was made in the neck extending slightly beyond the cephalic border of the submaxillary glands, which were freed in the midline by blunt dissection and retracted laterally. The sternohyoid muscle fibres were split in the midline and retracted laterally by a special retractor. The lateral lobes of the thyroid gland were exposed and gently displaced upwards and medially; care was taken to avoid damage to the recurrent laryngeal nerves. The parathyroid glands were removed with small adjacent portions of thyroid. All excised tissue was examined histologically to confirm that parathyroidectomy had been effectively carried out. When incomplete parathyroidectomy was confirmed (this occurred in 8% of animals), the animals concerned were excluded from further study. Fifty rats died immediately after, or within a few hours of, parathyroidectomy, in most cases from haemorrhage or anaesthesia, but the operative mortality decreased with experience. Tetany was not observed in any animals.

Blood Samples.—Blood was withdrawn under light ether anaesthesia from the internal jugular vein (Mouzas and Weiss, 1960) and transferred to siliconed tubes. The serum was separated within one hour of withdrawal: approximately 1 ml. of unhaemolysed serum was obtained for determination of alkaline phosphatase by the Folin-Ciocalteu reagent method of King and Armstrong (1934) and phosphorus by the method of King and Wootton (1956).

Parathyroid Hormone.—Parathormone was used in all experiments: dilutions were made in physiological saline, and final injection volumes in all cases were 2 ml.

EXPERIMENTS

1. Blood was withdrawn from 50 rats to establish basal values for serum alkaline phosphatase and phosphorus concentrations before parathyroidectomy.

2. Parathyroidectomy was carried out in 250 rats: blood was withdrawn from 50 on the 5th day, and 50 on the 8th day, another 50 on the 10th day, another 25 on the 12th day, another 25 on the 15th day, and another 50 on the 23rd day after operation: serum alkaline phosphatase and phosphorus concentrations were determined on each blood sample.

3. Parathyroidectomy was carried out in a further 245 rats, which were subsequently divided into five separate batches of 49. As a result of the observations made in experiment 2, the rats in four groups were injected respectively with 1, 10, 25, and 100 U.S.P. units of parathormone, all on the 14th day after parathyroidectomy: one group of 49 rats was not given parathormone and was used as a control. Blood samples were withdrawn 21 hours after the parathormone injections, and serum alkaline phosphatase and phosphorus concentrations were determined.

RESULTS

1. Before parathyroidectomy the mean values for serum alkaline phosphatase and phosphorus concentrations were 26.4 K.-A. units and 6.5 mg./100 ml. respectively (standard deviation 7.4 and 0.44) (Table I).

TABLE I.—Mean Serum Alkaline Phosphatase and Phosphorus Concentrations in 300 Rats Before and After Parathyroidectomy (only 25 Rats were Studied on the 12th and 15th Days Because the Values for the Two Days were Similar)

No. of Rats	Times of Samples	Alkaline Phosphatase (K.A. Units/100 ml.)	S.D.	Phosphorus (mg./100 ml.)	S.D.
50	Before operation	26.4	7.4	6.5	0.44
50	5 days after	18.7	5.4	7.1	1.20
50	8 " "	16.6	4.5	7.4	0.95
50	10 " "	35.8	12.5	7.4	0.52
25	12 " "	21.0	4.2	7.9	0.46
25	15 " "	21.2	4.4	7.9	0.38
50	23 " "	19.3	4.0	7.9	0.62

2. After parathyroidectomy the values for serum alkaline phosphatase and phosphorus fluctuated randomly and on the 5th day the mean serum alkaline phosphatase had decreased to 18.7 while the phosphorus had increased to 7.1; on the 8th day these values had become 16.6 and 7.4; on the 10th day 35.8 and 7.4; and on the 12th day 21.0 and 7.9. Thereafter, the mean values for serum alkaline phosphatase and phosphorus remained virtually unchanged up to 23 days after parathyroidectomy.

TABLE II.—Mean Values of Serum Alkaline Phosphatase and Phosphorus in 245 Parathyroidectomized Rats. One Group of 49 Rats Received No Parathormone and Served as a Control to the Other Groups which were Injected with Different Amounts of Parathormone

No. of Rats	Para-thormone (U.S.P. Units)	Alkaline Phosphatase (K.A. Units/100 ml.)	S.D.	Phosphorus (mg./100 ml.)	S.D.
49	None	19.8	4.1	7.9	0.5
49	1	27.0	4.6	7.4	0.12
49	10	32.2	2.6	7.9	0.36
49	25	37.5	4.8	8.0	0.65
49	100	43.3	5.2	9.2	1.2

3. When 1 U.S.P. unit of parathormone was injected 14 days after parathyroidectomy, the mean serum alkaline phosphatase and phosphorus values, 21 hours later, were respectively 27.0 and 7.4; when 10 units were given, the values were 32.2 and 7.9; when 25 units were

given, the values were 37.5 and 8.0; and when 100 units were given, the values were 43.3 and 9.2 (Table II).

DISCUSSION

No particular significance is attached to the change in the serum phosphorus concentrations in the experiments. It is believed, however, that the change in serum alkaline phosphatase concentrations observed in parathyroidectomized rats 14 days after operation after injection of parathormone affords a significantly sensitive method for detection of hormone. In view of the quantitative relationship between the administered dose of the hormone and the alkaline phosphatase, the technique may also provide a basis for the assay of parathyroid hormone and, moreover, it compares favourably with the method described by Davies *et al.* (1955), which required an average of 10 rats for each determination and detected only 2.5 U.S.P. units.

In experiments 1 and 2 (Table I) the animals were divided arbitrarily into five groups of 10 in five instances and five groups of five in two instances; and, since the standard deviation of the results of the alkaline phosphatase determination in each group did not exceed 0.46, reproducibility of results was considered to be adequate. In experiment 3 (Table II) the animals were similarly divided into seven groups of seven in five instances: the standard deviation of the results determined in each group did not exceed 0.52, so that reproducibility was considered to be adequate.

Parathyroidectomy in rats is a relatively simple procedure when a small portion of adjacent thyroid is removed at the same time and tetany is prevented by an adequate pre-operative calcium intake (Shelling, 1932).

SUMMARY

Parathyroidectomized rats respond to small doses of parathormone by a significant elevation of the serum alkaline phosphatase.

It is suggested that this affords a more sensitive index of parathormone activity than previously described methods.

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