The Activity *in vitro* of Growth Hormone in Reducing the Respiratory Quotient of Rat Diaphragm

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The administration to the rat under suitable conditions of crude anterior-pituitary extracts (Fisher, Russell & Cori, 1936), of extracts rich in growth hormone (Greaves, Freiberg & Johns, 1940) or of purified growth hormone (Greenbaum, 1953) can bring about a reduction of respiratory quotient of the animal as a whole. In important notes Recant (1952*a, b*) stated that effects of this sort could be induced *in vitro* by certain pituitary extracts, including some preparations of growth hormone, with isolated rat diaphragm suspended in glucose-containing buffer. We have undertaken the present investigation to ascertain whether this effect can be elicited with the purified preparations of growth hormone made in this Laboratory.

EXPERIMENTAL

Animals. Male albino rats of inbred laboratory strain, weighing 100–150 g. and maintained on a stock diet (Short & Parkes, 1949) were used. For the experiments with diaphragm food was withdrawn overnight and the animals were killed by decapitation.

Hormone preparations. Growth hormone was prepared from ox anterior-pituitary tissue, by a modification of the method of Wilhelmi, Fishman & Russell (1948). A crude sterile alkaline extract of ox anterior-pituitary tissue (Young, 1953) was used in some experiments. This preparation, referred to as FGS, was rich in growth hormone and also contained other anterior-pituitary factors. We also did experiments with two fractions of FGS kindly made available to us by Mr B. Ketterer. These fractions had been prepared by electrophoresis of FGS in barbital buffer at pH 8.6 and ionic strength 0.1 in a column of cellulose treated with ethanol and acetyl chloride, according to the method of Campbell & Stone (1956). The protein concentration of the fractions eluted from the column was determined by the method of Lowry, Rosebrough, Farr & Randall (1951). Under these conditions FGS separated into five peaks. The eluates corresponding to each peak were combined, dialysed exhaustively against distilled water and freeze-dried. F_3 and F_4 were the third and fourth of these fractions. The faster-moving component (F_3) possessed no growth-promoting activity whereas the slower-moving one (F_4) was highly active in this respect (see Table 3).

Determination of respiratory quotient *in vitro*. The first method of Dickens & Simer (1930) was followed. The buffer used was Ringer phosphate (Krahl, 1951) with the omission

of CaCl_2 , where necessary a 10% (w/v) solution of glucose being added to the buffer in such amount as to make a final glucose concentration of 0.2%.

In each experiment the procedure was as follows: the diaphragm was removed from the rat immediately after death, divided into halves and placed for 10–15 min. in ice-cold Ringer phosphate solution through which a stream of oxygen was passing. Each hemidiaphragm was then gently dried on filter paper, trimmed and put into the incubating medium. The hemidiaphragms were then allowed to respire at 37° for 2 hr., three groups of flasks being set up in each experiment: (1) with no glucose or hormonal preparation; (2) in the presence of glucose but with no hormonal preparation; (3) in the presence of glucose and of hormonal material. In some control experiments the hormonal preparation was replaced by an extract of rat-liver tissue prepared by the method used for obtaining the crude anterior-pituitary extract, and in other control tests a solution of rabbit-muscle tropomyosin (Bailey, 1948), in concentration comparable with that of the growth hormone, was used. The differences between the mean values for R.Q., over a period of 2 hr. under the different conditions, were determined and the probability (P) was calculated that the difference was due to chance.

Growth-promoting activity. Daily subcutaneous injections of growth hormone were given for 10 days to groups of approximately five rats which had been hypophysectomized about 2 weeks before the first injection. The animals were weighed daily before and during the experiment and the mean weight increase during the period of 10 days was calculated. In each experiment a group of control rats received treatment with 0.9% NaCl instead of growth hormone, and two groups received different doses of the Laboratory standard preparation of growth hormone (preparation 55, Table 3). The results are expressed in terms of the difference in mean weight between the control and experimental groups.

RESULTS

Table 1 indicates that the addition of a crude anterior-pituitary extract (FGS) *in vitro* reduces the R.Q. of the isolated diaphragm respiring in glucose under the conditions of our experiments. Since, in the absence of glucose, the R.Q. obtained was 0.711 ± 0.010 (mean value for 14 experiments) the reduction resulting from the addition of the pituitary extract in some experiments was to about the value to be expected in the absence of glucose. The crude pituitary extracts all possessed some R.Q.-lowering activity *in vitro*.

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When 0.2 mg. of purified growth hormone was added (Table 2) the mean values of the R.Q. were all below those in control experiments, significant differences between the effects of different batches of growth hormone being seen. Preparations 57 and 62 were hardly active at all, and the activity of preparation 61 was the only one whose effect in depressing R.Q. *in vitro* was shown to be statistically

highly significant. All these preparations of growth hormone possessed similar growth-promoting activities and were not significantly different in activity from the International Standard preparation of growth hormone (Table 3).

The clear-cut lack of relationship between growth activity *in vivo* and ability to reduce R.Q. *in vitro* is illustrated by the results with preparations

Table 1. *Effect of the addition in vitro of ox-pituitary preparations on the respiratory quotient of isolated rat diaphragm respiring in glucose solution*

Preparation added		No. of observations	R.Q.		
Description	Amount (mg.)		Mean ($\pm$ S.E.)	Difference between experimental and control values ($\pm$ S.E.)	Probability that difference is significant
Crude liver extract	0.5	9	0.848 $\pm$ 0.025	—	—
Crude pituitary extract no. FGS 308	0.5	7	0.730 $\pm$ 0.017	-0.118 $\pm$ 0.033	<0.01
FGS 310	0.5	8	0.708 $\pm$ 0.023	-0.140 $\pm$ 0.034	<0.01
F ₃	0.2	20	0.638 $\pm$ 0.042	-0.210 $\pm$ 0.049	<0.001
F ₄	0.2	19	0.641 $\pm$ 0.041	-0.641 $\pm$ 0.041	<0.001

Table 2. *Effect of different preparations of ox-pituitary growth hormone on the respiratory quotient in vitro of isolated rat diaphragm respiring in glucose solution*

Growth-hormone preparation added		No. of observations	R.Q.		
No.	Amount (mg.)		Mean ($\pm$ S.E.)	Difference between experimental and control values ($\pm$ S.E.)	Probability that difference is significant
(Control)	—	30	0.804 $\pm$ 0.014	—	—
57	0.20	7	0.786 $\pm$ 0.019	-0.018 $\pm$ 0.024	>0.5
60	0.20	18	0.735 $\pm$ 0.029	-0.071 $\pm$ 0.032	<0.05
61	0.20	11	0.660 $\pm$ 0.038	-0.144 $\pm$ 0.040	<0.01
62	0.20	9	0.750 $\pm$ 0.034	-0.054 $\pm$ 0.037	>0.1
60	0.002	4	0.812 $\pm$ 0.003	—	—
	0.020	3	0.793 $\pm$ 0.028	-0.011 $\pm$ 0.031	>0.5
	0.200	5	0.740 $\pm$ 0.023	-0.064 $\pm$ 0.027	<0.05
	2.000	4	0.682 $\pm$ 0.079	-0.122 $\pm$ 0.080	>0.1
(Tropomyosin)	2.000	5	0.810 $\pm$ 0.095	—	—

Table 3. *Growth-promoting activity, in hypophysectomized rats, of different preparations of growth hormone*

Growth-hormone preparation	No. of animals in each group	Increase in weight above that of controls	
		Amount (μ g./day) 47.5	Amount (μ g./day) 15
International Standard preparation	10	19.5 $\pm$ 2.8	13.0 $\pm$ 1.8
55 (Laboratory standard)	10	22.0 $\pm$ 1.9	15.0 $\pm$ 2.0
55	60	21.2 $\pm$ 1.5	13.4 $\pm$ 1.4
57	5	16.4 $\pm$ 4.7	4.5 $\pm$ 4.7
60	5	17.9 $\pm$ 2.2	12.3 $\pm$ 2.1
61	5	19.3 $\pm$ 3.2	9.0 $\pm$ 3.7
62	5	23.3 $\pm$ 3.3	11.2 $\pm$ 3.1
F ₃	5	—	-1.2 $\pm$ 1.2*
F ₄	5	—	13.4 $\pm$ 2.2*

* 20 μ g./day.

F_3 and F_4 . Both of these were active in reducing the R.Q. *in vitro* (Table 1) but only one (F_4) possessed growth-promoting activity.

Since some pituitary preparations with intermedin activity (Collip, 1938; Folley, 1955) have been found to possess also an R.Q.-lowering action, Dr H. B. F. Dixon kindly tested some of our preparations for melanocyte-stimulating activity in frogs and found no relationship between the small amount of intermedin-like activity observed and the R.Q.-reducing activity.

DISCUSSION

There is no doubt that the administration of crude pituitary extract and of purified growth hormone to the intact animal lowers the R.Q., but the physiological significance of the action of pituitary preparations in reducing the R.Q. *in vitro* is not certain. However, since we have preparations of growth hormone without significant effect on the R.Q. *in vitro* (Table 1), and other pituitary preparations deficient in growth-promoting activity but active in reducing the R.Q. *in vitro* (for example F_3 , Tables 1 and 3) there is no reason to suppose that the action *in vitro* is a property of growth hormone itself. This is in agreement with the conclusions of Recant (1952*a, b*).

SUMMARY

1. The effect of the R.Q. of isolated rat diaphragm respiring in glucose medium of the addition of

growth-promoting pituitary preparations *in vitro* was studied.

2. No relationship was found between growth-promoting activity in the hypophysectomized rat and R.Q.-depressing ability *in vitro*. The R.Q.-depressing factor appears not to be growth hormone.

We acknowledge the assistance of Miss Marjorie Thomas, who hypophysectomized the rats and also carried out some of the assays of growth hormone recorded here. The expenses of this investigation were partly defrayed from an expenses grant to one of us (F.G.Y.) from the Medical Research Council, for which we wish to express our thanks.

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Observations upon the Toxic Principle in the Seeds of *Dichapetalum toxicarium*

SEPARATION OF A LONG-CHAIN FATTY ACID CONTAINING FLUORINE

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The seeds of *Dichapetalum toxicarium* (also called ratsbane) have been known for long (Power & Tutin, 1906) to have toxic properties which bear a general resemblance to the effect of fluoroacetate found in the leaves of the South African plant *Dichapetalum cymosum* (Gifblaar), a potent cattle

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poison (Marais, 1944). Some of these seeds became available to us from Sierra Leone, by the kindness of Sir John Simonsen, F.R.S., and the Government Departments concerned (for a photograph of these seeds see Peters, 1954); and we have tried to isolate and characterize the active principle. In this paper, we describe the separation of a fraction containing a long-chain fluoro fatty acid. This work was