of the equations (4) to (17) can now be given. They are

$$\text{SGAG} = M[e^{-(x+2z)t} - e^{-(x+3z)t}], \quad (22)$$

$$\text{SGA} = M[e^{-(x+z)t} - e^{-(x+2z)t}], \quad (23)$$

$$\text{SG} = M[e^{-xt} - e^{-(x+z)t}], \quad (24)$$

$$\text{S} = M[1 - e^{-xt}], \quad (25)$$

$$\text{GAGAG} = [Mx/(x+z-y)] [e^{-(y+3z)t} - e^{-(x+4z)t}], \quad (26)$$

$$\text{GAGA} = [Mx/(x+z-y)] [e^{-(y+2z)t} - e^{-(x+3z)t} - e^{-(y+3z)t} + e^{-(x+4z)t}], \quad (27)$$

$$\text{AGAG} = M \left[\frac{z^2 + xy}{z(2z+x-y)} \cdot e^{-(y+2z)t} - \frac{xy}{z(z+x-y)} \cdot e^{-(y+3z)t} - \frac{(x+z)(z-y)}{(z+x-y)(2z+x-y)} \cdot e^{-(x+4z)t} \right]. \quad (28)$$

The other formulae are complicated and considerably more cumbersome to use in this form than those given above. Consequently, it was found better not to use the integrated equations for the remaining peptides and amino acids, but to calculate the coefficients of each exponential numerically for each value of z chosen with the observed numerical values $x=0.0056$, $y=0.0137$ and with M as 1. This was done by making use of equations (19) and (20). Thus in each equation of type (19), when x , y and z are given, the values of

$$A, \alpha, B, \beta, \dots, \lambda$$

are known. Hence by substituting these values in (20) the numerical value of each coefficient in (20) is obtained. Thus the coefficients for the tripeptides can be obtained from the known values for the tetra- and higher peptides, and similarly from these

the dipeptide coefficients can be found and, finally, those for G and A .

It should be noted from equation (25) that, as $t \rightarrow \infty$, $S \rightarrow M$. Similarly, it is obvious that, as $t \rightarrow \infty$, $A \rightarrow 2M$, $G \rightarrow 3M$. Hence the accuracy of the calculations to obtain values for A and G , in the manner described in the previous paragraph, may be checked by letting $t \rightarrow \infty$. The formulae for SGA and SG , which are fairly simple, enable a good idea of the likely value of z to be found. Further values of z around this value were then tried and the one that gave the best numerical fit was chosen. This was $z=0.0500$.

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The Effect of Growth Hormone upon Cell Division and Nucleic Acid Synthesis in the Regenerating Liver of the Rat

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(Received 27 December 1956)

Growth hormone markedly increases the low mitotic activity in the adrenal cortex of the hypophysectomized rat to levels above those found in the intact animal (Cater & Stack-Dunne, 1953). A single dose of 1 or 5 mg. of growth hormone injected into rats hypophysectomized 10–21 days previously increased the number of mitoses in the adrenal cortex in 4–8 hr. (Cater & Stack-Dunne, 1955). As a method for the study of the mode of action of growth hormone upon mitosis this preparation presents many difficulties. Holmes & Mee (1954, 1955) have reported upon nucleic acid synthesis, and

Cater, Holmes & Mee (1956) have published data on the rate of mitosis, deoxynucleic acid (DNA) and ribonucleic acid (RNA) synthesis in rat liver after partial hepatectomy. It therefore appeared of interest to investigate the effect of growth hormone upon regenerating liver. In the early stages of regeneration of liver, the various processes of the mitotic cycle can be studied separately. An increase of RNA synthesis is obvious at 11 hr. after partial hepatectomy; the rate of DNA synthesis begins to increase at about 12 hr., and at about 24 hr. after the operation is 10 times the normal rate. Mitotic activity, which is still near zero at 22½ hr., rises to 30–40/1000 cells at 27–28½ hr. after hepatectomy.

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Mention has been made that growth hormone has an effect upon this system in a brief preliminary report (Cater, 1957).

METHODS

Animal technique. Male black-and-white hooded rats (about 300 g. in wt.) of an inbred strain were anaesthetized with ether and two lobes of the liver removed by the method of Higgins & Anderson (1931). Before the operation they were fed on a diet supplemented with raw meat and green vegetable for 2 days, and after the operation they were kept warm and fed on carrots or bread and sugar. The rats were operated on in pairs, one being the control, the other being injected subcutaneously with growth hormone (2 mg. at operation, 4 mg. at approx. 8 hr. and 4 mg. at 5-6 hr. before killing). All were injected subcutaneously with 50 μ C of 32 P 3 hr. before death, and were killed at various times between 22 and 30 hr. after hepatectomy. The growth hormone was prepared by Mr B. R. Slater of the Department of Biochemistry, Cambridge, a modification of the method of Wilhelmi, Fishman & Russell (1948) being used.

Mitotic counts. At death a representative, thin slice of liver was fixed in Suza fluid. Paraffin sections 6 μ thick were prepared and stained with haematoxylin and eosin. A square aperture was placed in the focusing eye-piece of the microscope. This gave a field 110 μ square with the 2 mm. objective. The mitoses present in 200 fields were counted. The number of liver-cell nuclei in every tenth field was counted. The results are given as a mitotic index, i.e. the number of mitoses/1000 cells. As 200 fields represent 4000-5000 cells the mitotic index is dependable. The phases of mitosis were recorded.

Nucleic acid estimations. The inorganic phosphate was estimated on a small sample by the isobutanol extraction method of Weil-Malherbe & Green (1951). The method of Schmidt & Thannhauser (1945) was used to separate and estimate the phosphorus of DNA and RNA from 2 g. of liver. Counts of 32 P in all the fractions were made with a liquid-dipping counter. The activities of the nucleic acids are expressed as:

$$\frac{\text{Specific activity of DNAP (or RNAP)}}{\text{Specific activity of inorganic P}} \times 20000.$$

The factor 20000 was chosen as the usual value obtained for the specific activity of the inorganic fraction was about 20000 counts/min./0.1 mg. of P.

RESULTS

Mitotic counts. The effect of growth hormone was to increase the rate of cell division after partial hepatectomy (Fig. 1). The time of onset of mitosis in controls was between 23 and 24 hr. after partial hepatectomy. In rats treated with growth hormone mitosis had begun at 23 hr. and was significantly greater than in the controls. At 24½, 25 and 25½ hr. the growth-hormone-treated rats showed more than twice as many cells in mitosis as did the controls, and at 26 hr. there were nearly three times as many. A higher rate of mitosis persisted in the growth-hormone-treated rats until at least 30 hr. In each pair of rats the one receiving growth hormone always showed a higher mitotic count than the control.

The statistical analysis is given in detail in the legend to Fig. 1. It will be noted that the means of the mitotic index of the growth-hormone-treated rats are significantly greater than those for the control rats at times from 23 to 30 hr., except at 24½ hr.

Fig. 1. Mitoses/1000 cells in regenerating liver when the rats were treated with growth hormone (□), and in control rats (○). Means, standard errors of means and the numbers of rats are shown. Values for Student's *t* test with the Bessel correction are: 23 hr., *t*=2.8, *n*=6, *p*<0.05; 24 hr., *t*=3.9, *n*=5, *p*≈0.01; 24½ hr., *t*=1.54, *n*=4, *p*=0.2; 25 hr., *t*=4.3, *n*=6, *p*<0.01; 25½ hr., *t*=4.8, *n*=3, *p*<0.02; 26 hr., *t*=7.1, *n*=4, *p*<0.002; 27 hr., *t*=3.05, *n*=4, *p*<0.05; 28½ hr., *t*=2.1, *n*=4, *p*=0.1; 30 hr., *t*=2.1, *n*=4, *p*=0.1. ■, ○, Single rats.

Fig. 2. Mitoses in regenerating liver when the rats were treated with growth hormone (□) compared with all previous control rats (○); means, standard error of means and the numbers of rats are shown. Statistical analyses: 23 hr., *t*=3, *n*=8, *p*<0.02; 24 hr., *t*=4.4, *n*=19, *p*<0.001; 24½ hr., *t*=1.54, *n*=4, *p*=0.2; 25 hr., *t*=4.3, *n*=6, *p*<0.01; 25½ hr., *t*=5, *n*=15, *p*<0.001; 26 hr., *t*=5.8, *n*=17, *p*<0.0001; 27 hr., *t*=2.1, *n*=42, *p*=0.05; 28½ hr., *t*=3, *n*=7, *p*=0.02; 30 hr., *t*=3.45, *n*=15, *p*=0.002. ■, ○, Single rats.

where p is 10%. Analysis of the data, by comparing each growth-hormone-treated rat with its own control rat, did not yield any further information.

It was noted in this particular series of experiments that the mitotic counts of the control rats were rather low compared with our previous experience. When, however, the means of the mitotic indices of the growth-hormone-treated rats were compared with the pooled controls of all previous experiments (Fig. 2), the increased mitosis due to growth hormone is still highly significant. The details of the statistical analysis are given in the legend to Fig. 2.

The growth-hormone-treated rats showed certain cytological changes in the liver cells when compared with the control rats. The chromatin of the nuclei was more heavily stained and many of the mitotic figures were large.

The phase counts showed a normal distribution of the phases of mitosis in the growth-hormone-treated rats. In this way the action of the growth hormone is quite unlike that of colchicine and there is no suggestion that it acts merely by increasing the time taken by each cell to complete mitosis. In earlier experiments colchicine was given to animals 3 hr. before killing, and it was found that all the dividing cells were in arrested metaphase, with the chromosomes scattered throughout the cytoplasm. There were few cells in prophase and none in anaphase or telophase.

The difference in the action of the two substances was obvious when colchicine and growth hormone were given to the same rat; the phase distribution typical of colchicine was then found and very many 'exploded metaphases' were seen.

Rate of synthesis of deoxyribonucleic acid fraction. The DNA fraction obtained from the Schmidt-Thannhauser separation was taken without further purification. In previous experiments (Mee, 1956) where both ^{32}P and ^{14}C were used, the nucleic acids were first extracted with saline and, after separation, the DNA was further purified to remove all contaminating protein and RNA. In experiments on both normal and regenerating liver, similar results were obtained to those on the Schmidt-Thannhauser DNA fraction and there was no suggestion that any effects obtained were on any contaminant and not on DNA itself. It was therefore thought unnecessary to purify further the DNA fraction in this series of experiments.

The results given in Figs. 3 and 4 show that growth hormone increased the rate of DNA synthesis in regenerating liver during the first period of rapid DNA formation, which occurs in rats between 21 and 26 hr. after partial hepatectomy.

As the ^{32}P is always injected 3 hr. before killing, the radioactive counts of DNAP plotted in these figures at points corresponding to the times of

killing represent a summation of events that have taken place during that interval.

As each treated rat had its own control it is legitimate to plot the ratio of DNA synthesis in the growth-hormone-treated rats to that of their controls (Fig. 4).

Fig. 3. Rate of DNA synthesis in rat liver after partial hepatectomy as indicated by the uptake of ^{32}P given 3 hr. before death in rats treated with growth hormone ($\square$) and in control rats ($\circ$). Means and standard errors of the means are plotted and the numbers of rats indicated. $\blacksquare$, Single rat.

Fig. 4. Rate of DNA synthesis in the growth-hormone-treated rat expressed as a percentage of that in the control rat; means and standard errors of the means are plotted for the groups of paired rats. Statistical analysis shows that results at 25 and 27 hr. are significant at the 2% level and at 23 hr. at the 5% level. $\times$, One pair of rats.

The peak of the DNA synthesis rate, which occurs in control animals at 24 hr., is higher and more prolonged in the growth-hormone-treated rats. At 28½ hr., when the rate of synthesis is falling in both control and treated animals, there is no significant difference between the two.

Uptake of ^{32}P into ribonucleic acid fraction. Since we were chiefly concerned with mitotic counts and with DNA synthesis, we did not make any attempt to separate the components of the Schmidt-Thannhauser 'RNA' fraction, but measured the ^{32}P uptake into the fraction as a whole. Schmidt & Thannhauser realized that inorganic P derived from protein-bound P was also contained in this

solution; Davidson & Smellie in 1952 showed the presence of five other phosphorus-containing compounds and recently Hutchison *et al.* (1956) have detected others, including phosphoserine and inositol mono- and di-phosphates. Some of these contaminants have a far more rapid ^{32}P uptake than RNA itself. The results in Fig. 5 show that the rate of ^{32}P incorporation into this fraction was higher in hormone-treated rats than in controls, although it is not possible to say which of the chemical substances present was responsible.

The incorporation was always higher in the growth-hormone-treated rat than in the control rat of the pair, with the exception of those killed at 30 hr. It is legitimate to use the ratio of 'RNA' synthesis in each pair of rats for statistical analysis. The results shown in Fig. 6 indicate that 'RNA' synthesis is higher in the growth-hormone-treated rats from 23 to 27 hr. and then falls until it reaches equality at 30 hr. For the whole series, the mean value for the treated rats is $123 \pm 4\%$ ($n = 29$) of the value for controls; the difference is highly significant.

DISCUSSION

Treatment of rats with growth hormone after partial hepatectomy causes an increased mitotic peak, which starts slightly earlier and lasts longer than in the controls. There is also a higher peak of DNA synthesis, which returns to control levels at 28½ hr. Since DNA synthesis precedes actual mitosis by some hours, the DNA synthesis is usually found to be falling at a time of high mitotic activity. In growth-hormone-treated rats the mitotic peak is prolonged and at 26 hr. a high DNA synthesis and a high mitotic count are often found together.

There is also a small but significant increase of ^{32}P incorporation into the Schmidt-Thannhauser RNA fraction.

We believe that the effect of growth hormone described here is a real one and that growth hormone is not acting like colchicine to give falsely high mitotic counts by prolonging the time taken by cells to complete mitosis. The cytological appearances and normal phase counts found after treatment with growth hormone are strong evidence against a colchicine-like effect. The greatly increased DNA synthesis also suggests a real increase in mitosis and is not produced by giving colchicine.

Our experiments were designed upon the hypothesis that growth hormone is the hormone responsible in part or whole for the regeneration of the liver after the stimulus of partial hepatectomy. The dosage of growth hormone was therefore deliberately high in order to be greater than the output of the hormone from the rat's own pituitary. The timing of the doses was such as to ensure demonstrating an effect if one were produced.

Fig. 5. Rate of ^{32}P uptake into the Schmidt-Thannhauser 'RNA' fraction in rat liver after partial hepatectomy. Rats treated with growth hormone, □; control rats, ○. Means and standard errors of the means are plotted and numbers of rats indicated. ■, ●, Single rats.

Fig. 6. Rate of 'RNA' synthesis (Schmidt-Thannhauser fraction) in the growth-hormone-treated rat expressed as a percentage of that in the control rat of the pair. Means and standard errors of the means are plotted for the groups of paired rats. Statistical analysis shows that results at 23 hr., 24½ hr., 25 hr., 26 hr. and at 27 hr. are significant at the 5% level. ×, One pair of rats.

The work of Stefano & Diermeier (1956) on the effect of growth hormone upon the development of polyploidy in the liver is in keeping with the hypothesis that growth hormone is important in the growth of the liver. Proof of the hypothesis requires work on liver regeneration in hypophysectomized rats. Some preliminary experiments which we have performed gave no positive result, but investigations are being continued.

It is also of interest to enquire whether growth hormone exerts its effect (a) in the events which precede DNA synthesis, (b) during DNA synthesis, or (c) during the mitotic act. In our experiments growth hormone was used in effective concentration during all of these significant periods. The results would suggest that the effect is upon events in stage (a) or (b), or possibly all three. This problem is capable of experimental investigation and further work is in progress.

Another problem for solution is whether growth hormone acts directly upon regenerating liver, or whether its effect is exerted through some other hormone or combination of hormones. For example, the close co-operation between growth hormone and insulin has been reviewed by Young (1953).

SUMMARY

1. Rats after partial hepatectomy were treated with subcutaneous injections of growth hormone, 2 mg. at 0 hr., 4 mg. at about 8 hr. and 4 mg. at 4–6 hr. before killing. These were compared with control rats in regard to rates of mitosis, deoxy-nucleic acid synthesis and ribonucleic acid synthesis from 22 to 30 hr. after partial hepatectomy.

2. The wave of mitosis starts slightly earlier (by about 1 hr.), and is two to three times higher in the growth-hormone-treated rats.

3. The rate of deoxynucleic acid synthesis shows a higher and more prolonged peak in the growth-hormone-treated rats.

4. The rate of ^{32}P incorporation into the Schmidt-Thannhauser ribonucleic acid fraction is higher during the peak of synthesis in the growth-hormone-treated rats.

The authors wish to express their thanks to Professor J. S. Mitchell, F.R.S., for his constant advice and encouragement, and to Professor F. G. Young, F.R.S., for his generous gift of growth hormone and for his interest in the project. Mr Basil Slater very kindly prepared the growth hormone and Miss N. C. Burr prepared all the sections.

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L-Cystathionine in the Urine of Pyridoxine-deficient Rats

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(Received 26 July 1956)

Some time ago, when a study was made of the excretion of taurine in rats reared on a diet deficient in pyridoxine, a new ninhydrin-positive spot was seen on two-dimensional paper chromatograms which was absent from normal rat urine (Blaschko, Datta & Harris, 1953) and which was provisionally

identified as phosphorylethanolamine. When in the present series of experiments this spot was again noticed it was decided to characterize the compound more fully.

METHODS

Animals. Two different strains of rats were used: Wistar albinos obtained from the Anatomy Department, Birmingham University, and hooded rats of the Lister strain, which

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