the template hypothesis of protein synthesis, which has been recently established by direct experiments with synthetic RNA or polyribonucleotides, a large number of amino acids should 'at one stroke' be incorporated in the protein. One should therefore expect to detect 'pools' of many free amino acids, and these pools should alter markedly at different phases of protein biosynthesis. (On the other hand, HERRMANN et al.[3] have found evidence of embryonic protein biosynthesis from precursors bigger than amino acids.) A sufficient number of amino acids have been detected only in a few cases, e.g. in echinoid embryos.

We therefore performed acid hydrolysis of adult *Limnaea* (proteins) and detected only six to eight amino acids (Figure 2). However, prolonged (16 h) acid hydrolysis can destroy two particular amino acids[4]. Therefore, at most, the adult *Limnaea* have only ten amino acids. Direct crushing of bigger snails had of course revealed a streaky band of weak colour extending up to higher regions[1] of Rf value. In the present investigation, we once found such an effect even in a small snail (0.50 cm).

The small number of free amino acids is thus partly explained but it is surprising that there are only one or two free amino acids even in the mature egg where the embryo has been largely formed[5].

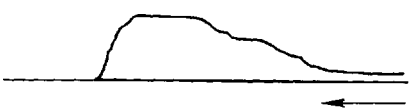

Fig. 1

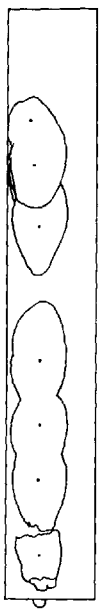

Fig. 2

Résumé. De nouvelles recherches ont amené les auteurs à constater l'existence de seulement 2 ou 3 acides aminés dans les œufs de la Limnée (Gastéropode), un jour avant l'éclosion. Ces acides sont très semblables à ceux que l'on trouve chez les jeunes escargots. Dans les produits hydrolytiques des protéines de l'escargot adulte, on constate la présence de 9 acides aminés.

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Research and Training School and Gerontology Unit, Indian Statistical Institute, Calcutta (India),
October 16, 1962.

[3] H. HERRMANN, in *Fundamental Aspects of Normal and Malignant Growth* (Elsevier, 1960), p. 497.
[4] *Biosynthesis of E. Coli* (Carnegie Institution of Washington Publications 1955), p. 27.
[5] We take this opportunity of thanking Dr. P. R. PAL, Dr. J. GHOSH, and Mr. K. K. BOSE for their kind help.

The Effect of Radiation on the Mitosis-Stimulating Activity of Hepatectomized Rat Serum

It has been demonstrated that in the serum of mammals there exists a factor both stimulating and inhibiting mitosis. FRIEDRICH-FREKSA and ZAKI[1] have produced cell division in the liver tissue by administering serum from rats whose livers were just regenerating after partial hepatectomy. ADIBI et al.[2] have demonstrated a considerable increase in the liver mitotic index after having injected serum from the hepatic vein of a hepatectomized rat into another partially hepatectomized rat.

In our experiments we have been studying the effect of X-radiation on the mitosis-stimulating factor in the serum of partially hepatectomized rats. There is still much discussion about the significance of the humoral factor responsible for the lowering of the mitotic activity following irradiation, as well as about the so-called distant effect of radiation, but no conclusive explanation has been established as yet (BACQ and ALEXANDER[3]).

In our experiments we used white Wistar rats, each weighing about 200 g. Partial hepatectomy was performed according to the technique of HIGGINS and ANDERSON[4]. Each group of experimental animals was irradiated 20 h after hepatectomy with a dose of 700 r (180 kV, 15 mA, dose rate 60 r per min, 0.5 mm Cu filter). 24 h after hepatectomy, blood was drawn with a syringe from the hepatic vein below the diaphragm of the animals. The serum was then separated from this blood by centrifugation. The mitosis-stimulating factor was tested on another group of partially hepatectomized animals in such a manner that 1.5 ml of serum was injected intraperitoneally 24 h after operation. Colchicine (0.1 mg per 100 g, Merck) was administered subcutaneously to the rats 18 h after the injec-

The mitotic index and standard error in regenerating liver

Rats were injected with:	Number of animals	Mitotic-index
1.5 ml of physiologic saline	3	1.0 ± 0.34
1.5 ml of serum from irradiated rats	5	3.8 ± 0.21
1.5 ml of serum from non-irradiated rats	5	9.5 ± 0.66

[1] H. FRIEDRICH-FRESKA and F. ZAKI, Z. Naturforsch. *9* b, 394 (1954).
[2] S. ADIBI, K. E. PASCHKIS, and A. CANTAROW, Exper. Cell Res. *18*, 396 (1959).
[3] Z. M. BACQ and P. ALEXANDER, *Fundamentals of Radiobiology*, Chapter 18 (Pergamon Press, Oxford 1961).
[4] G. M. HIGGINS and R. M. ANDERSON, Arch. Pathol. *12*, 186 (1931).

tion of serum. The rats were sacrificed 50 h after hepatectomy (26 h after the injection of serum). Paraffin sections were made, stained with hematoxylin-eosin, and the mitoses were then counted in 100 fields (5000 cells). The mitotic index (stathmocinetic index) was expressed as the number of mitoses per 100 cells.

From the results of our experiments (Table), it may be assumed that intraperitoneal injections of serum from partially hepatectomized rats have a stimulating effect on mitosis in the regenerating livers of all animals. The rise in the mitotic index was, however, lower in all those cases where the donor rats had been irradiated.

It may be preliminarily concluded that radiation reduces the mitosis-stimulating activity of serum. It is quite possible that the amount of a mitosis-stimulating agent is reduced by radiation, or that the amount of a mitosis-inhibiting agent—whether specific or non-specific—is increased as a result of radiation.

Zusammenfassung. Der Mitoseindex in der Rattenleber wird durch Injektion von Serum hepatektomierter Ratten beträchtlich gesteigert. Werden die Donatortiere mit 700 r bestrahlt, dann ist dieser Effekt signifikant reduziert.

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ACTH Antibodies and their Use for a Radioimmunoassay for ACTH

Until recently no conclusive evidence of specific antibodies to ACTH has been obtained because of the lack of precipitation of the ACTH-antibody complex[1]. The first demonstration for the antigenicity of ACTH was presented with the use of an haemagglutination technique[2,3]. Using a modification of the Yalow and Berson competitive inhibition technique[4] further evidence is presented for the antigenicity of ACTH. Details of the binding between ACTH and antibody are given.

The development of a sensitive method for the determination of circulating ACTH has long been awaited. The immunoassay presented here is based on a modification by Hales and Randle[5] of the insulin radioimmunoassay of Yalow and Berson[4]. It allows the detection of as little as 100 μμg ACTH in 0.1 ml and makes possible the determination of ACTH in normal human plasma as well as in plasma of patients whose ACTH level has been lowered by large doses of cortisol or derivatives.

Material and Methods. ACTH: Pure porcine A_1 ACTH was a gift from Dr. H. B. F. Dixon of the Department of Biochemistry of the University of Cambridge. It was obtained by a modification of the method of Dixon and Stack-Dunne[6] and found to be chromatographically pure.

ACTH-I^{131}: ACTH was labelled with I^{131} by an adaptation of the method developed by Hales and Randle[5] for insulin (on the principle given by McFarlane[7]). 50 μg ACTH in 10 μl pH 1.8 glycine-HCl buffer was incubated for 5 min with a mixture of 20 μl ICl and 4 to 12 mC NaI^{131} in 50 μl. It was then dialysed for 24 h against five changes of 0.01 *N* NH_4OH and purified on a cellulose column[4] using 15% human albumin as eluant. The specific activity obtained varied between 4 and 10 mC per mg ACTH.

Antibodies: One guinea-pig was immunized with pure porcine ACTH (7 mg in 5 injections) and two guinea-pigs with a commercial preparation of ACTH (75 international units in 5 injections). The animals were not adrenalectomized. For immunization these hormones were emulsified in complete Freund's adjuvant.

Anti-γ-globulin: Rabbit anti-guinea-pig γ-globulin was prepared by injecting rabbits with a preparation of crude guinea-pig γ-globulin emulsified with complete Freund's adjuvant. The guinea-pig γ-globulin was obtained by precipitation of 2 volumes of normal guinea-pig serum with one volume of saturated $(NH_4)_2SO_4$.

Buffers: Dilutions were made in 0.04 *M* phosphate buffer pH 7.4 containing 1/4000 merthiolate and 2 mg per ml

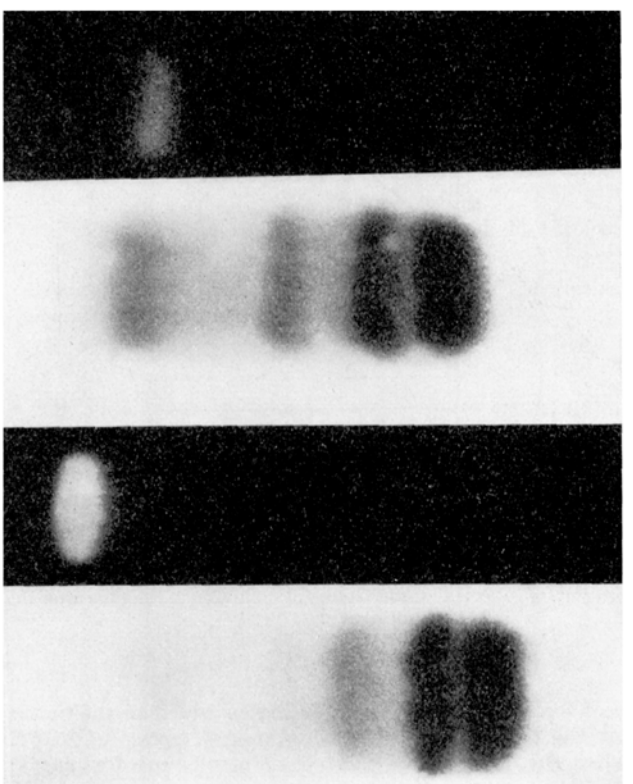

Fig. 1. Paper electrophoresis of antiserum (above) and normal serum (below) incubated for 24 h at 0° with ACTH-I^{131}. The electrophoresis was done in veronal buffer pH 8.6, 0.1 ionic strength at +4° for 15 h at constant voltage 4 V per cm. The main peak of radioactivity shown by contact photography moved with the γ-globulins in the incubation of the antiserum, whereas it remained at the origin in the case of normal serum.

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