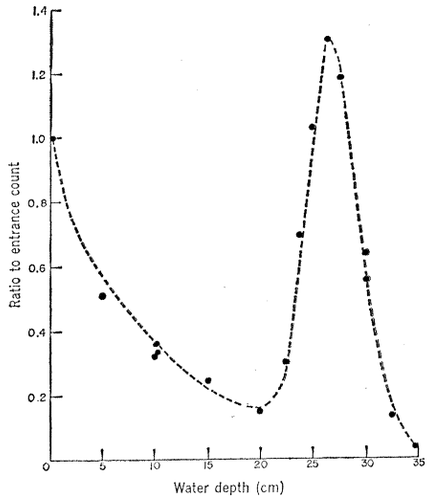

Fig. 1. The relative yield of positron emitters as a function of depth for 100-Mev negative pions in water.

The beam momentum at entrance is in the tail of the $T = 3/2$, $J = 3/2$ pion-nucleon resonance from which scattering and reactions are strong. As the energy becomes degraded, the yield from this resonance decreases. This effect is proposed as an explanation, by means of reactions (*1–4*) for the shape of the range curve for small amounts of absorber.

The plot of positron yield (Fig. 1) is similar in shape to the π^- depth-dose or Bragg curve in the region near the stopping volume. Hence, the induced positron activity can be used as a measure of the depth-dose. Near the beam entrance, the positron yield is enhanced by processes similar to Eq. 3 which do not give a large contribution to the dose, and in this region the activity does not correspond directly to the energy deposited. However, the deep valley between the two regions permits rather simple corrections for obtaining the dose.

The width of the peak in Fig. 1 is due to uncertainties in the pion range (the positrons have a range of less than 0.5 cm). The natural straggling of the pion beam has been calculated by Curtis and Raju (*5*) to be about 0.6 cm. The standard deviation of the peak is about 2.25 cm, which can be accounted for by a spread of momentum of about ±4 percent. When considered with this large effect, the other uncertainties are negligible.

The measurements indicate that the Bragg peak positron yield is about 1.2 percent of the total incident pions. This is a sufficiently large production cross section to get a good initial picture of the profile of stopped pions within a patient at a minimum dose to the patient, and should thus provide an accurate method of adjusting depth dosage with negative pions for therapy.

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References and Notes

1. P. H. Fowler and D. H. Perkins, *Nature* **189**, 524 (1961).
2. L. Rosen, *Nucl. Appl.* **5**, 379 (1968).
3. M. P. Guthrie, R. G. Alsmiller, Jr., H. W. Bertini, *Nucl. Inst. Meth.* **66**, 29 (1966).
4. Work performed at the Space Radiation Effects Laboratory, Newport News, Va.
5. S. B. Curtis and M. R. Raju, *Univ. Calif. Rad. Lab. Publ. No. 17606* (1967), p. 108.

13 April 1970

Prolactin Localization in the Primate Pituitary by Immunofluorescence

Abstract. *Cells which contain prolactin were clearly distinguished from those which contain growth hormone in adult monkey pituitary glands by means of histologic and fluorescent antibody techniques. The results indicate that in primates, as well as in other mammals, prolactin is immunochemically distinguishable from growth homone.*

Although cells containing prolactin (PRO) have been localized by immunofluorescence in the pituitary gland of various mammalian species such as the rat, ox, sheep (*1*), and in certain species of fishes (*2*), this has not yet been accomplished in primate pituitaries. The existence of PRO as a hormone separate from growth hormone (GH) in the primate pituitary has been questioned since it was demonstrated that human GH and monkey GH possessed biologic properties of PRO (*3*). Furthermore, the isolation and purification of a primate PRO free of GH activity has not been achieved. There is, however, some evidence which suggests that in the primate pituitary PRO may be produced by a cell type distinct from that which produces GH, as is thought to be the case in other mammals. Two separate acidophilic cells can be tinctorially distinguished in both monkey (*4*) and man (*5, 6*), one being orangeophilic and thought to secrete GH and the other being carminophilic, and thought to secrete PRO. By means of immunofluorescence with antiserum to human GH, it was found that only the orangeophils fluoresced in human pituitaries obtained from females in the latter half of pregnancy or 3 days after giving birth, whereas the carminophils, which are so numerous during these periods, did not fluoresce (*5*). These results tend to support the concept that a primate PRO may be secreted by the carminophils and may, therefore, exist as a separate hormone in the primate pituitary. We now report the findings of our immunological approach to this problem.

Antiserum to highly purified ovine PRO (SPRO) (NIH S-8) was prepared by the immunization of guinea pigs and was absorbed to remove any antibodies to serum proteins and ovine GH. When this antiserum was tested by the agar diffusion technique of Ouchterlony (*7*) against pituitary extracts from two rhesus monkeys which were pregnant for approximately 5 months and from two which were lactating for 3 and 6 weeks respectively, a precipitin line developed between the antiserum and each of the pituitary extracts. Each of these lines gave a reaction of partial identity with SPRO in an adjacent well, indicating that the reacting substance in these extracts is immunochemically related to SPRO. The antiserum showed no reaction with various concentrations of human GH.

For the immuno-histochemical studies, pituitaries were obtained from six adult monkeys, including two cynomolgus females, three rhesus females of which one was lactating normally for 6 weeks, and one rhesus male. The pituitaries were fixed in 10 percent neutral buffered formalin, then embedded in paraffin. Sections from all of these pituitaries were examined by the fluorescent antibody technique of Coons (*8*). The identical sections were also stained by Brookes' technique (*9*) which differentiated two types of acidophils, namely the carminophils, which were dark red in color, and the orangeophils, which stained a yellowish orange. The carminophils were of two general types,

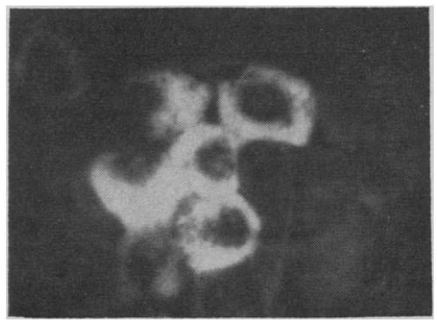

Fig. 1. Photomicrograph of a section of the anterior pituitary of an adult female rhesus monkey treated with guinea pig antiserum to SPRO followed by application of fluorescein-conjugated globulin prepared from antiserum to guinea pig globulin. The group of fluorescent cells in the center of the field as well as the weakly fluorescent cell in the upper left hand corner of the field are carminophils; the nonfluorescent cells to the right of center are all orangeophils. Other nonfluorescent cells in this field are either basophils or chromophobes. Magnification, ×1000.

consisting of either large cells frequently found in groups or clusters, or relatively small cells randomly distributed and occasionally present near the periphery of the section. The orangeophils were similar in size to the small carminophils and were not found in groups or clusters.

The orangeophils considerably outnumbered the carminophils in all but two female pituitaries, one of which was the lactating female, wherein nearly three-fourths of all cells were carminophils. The well-known function of prolactin during lactation (*10*) in all likelihood accounted for the large number of carminophils observed in the pituitary from the lactating animal. Since the state of the reproductive cycle in the other female monkeys was unknown, correlations could not be made with the relative numbers of carminophils present in their pituitaries. There was little doubt, however, that the pituitary of the lactating monkey showed the greatest absolute as well as relative numbers of carminophils.

The indirect technique was used in the fluorescent antibody studies, in which the same guinea pig antiserum to SPRO was employed as was used in the agar diffusion studies, followed by the application of fluorescein-conjugated globulin prepared from rabbit antiserum to guinea pig globulin (Nutritional Biochemical Corporation). The yellow-green fluorescence was clearly localized within both large and small types of cells (Fig. 1) which were identified as carminophils in the same section stained by Brookes' technique. Fluorescence did not localize within the orangeophils, basophils or chromophobes. Conversely, when guinea pig antiserum to human GH was used, followed by application of the same fluorescein-conjugate as used above, only the cells identifiable as orangeophils fluoresced. The latter was demonstrated in sections from three different pituitaries including one from a male and two from female monkeys. Various tests were performed as controls for the specificity of fluorescence, among which were: (i) incubation of the guinea pig antiserum to SPRO with unconjugated antiserum to guinea pig gamma globulin before its application to the section, followed by application of the fluorescein-conjugate; (ii) examination of the unstained, untreated section; and (iii) treatment of the section with guinea pig antiserum to human GH followed by application of the fluorescein-conjugate used above. None of the control tests showed fluorescence in the carminophils. The last of these procedures showed fluorescence only in the orangeophils and served as an experimental procedure, as previously mentioned, as well as a control.

Since only the carminophils fluoresced when pituitaries were treated with antibodies to SPRO and only the orangeophils fluoresced when treated with antibodies to GH, the results indicate that there is a substance in the primate pituitary that is immunochemically both related to SPRO and distinguishable from primate GH. This substance is presumably primate PRO. Our findings by no means disregard the biologic properties of PRO associated with preparations of purified human GH and monkey GH (*3*). These properties may well be intrinsic to the primate GH molecule, but the results of our investigation strongly suggest the existence of a separate PRO molecule in primate pituitaries.

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References and Notes

1. R. Nayak, E. E. McGarry, J. C. Beck, *Endocrinology* **83**, 731 (1968)
2. E. W. Emmart, *Gen. Comp. Endocrinol.* **12**, 519 (1969).
3. W. R. Lyons, C. H. Li, R. E. Johnson, in *Program of the Forty-Third Meeting of the Endocrine Society (June 1961)* (Thomas, Springfield, Ill., 1961), Abstract No. 7; W. D. Peckham, J. Hotchkiss, E. Knobil, C. S. Nicoll, *Endocrinology* **82**, 1247 (1968).
4. A. B. Dawson, *Anat. Rec.* **102**, 103 (1948).
5. M. Heylant and J. L. Pasteels, *Exp. Pathol.* **3**, 250 (1967).
6. L. G. Goluboff and C. Ezrin, *J. Clin. Endocrinol.* **29**, 1533 (1969).
7. O. Ouchterlony, *Acta Pathol. Microbiol. Scand.* **32**, 231 (1953).
8. A. H. Coons, E. H. Leduc, J. M. Connoly, *J. Exp. Med.* **102**, 49 (1955).
9. L. D. Brookes, *Stain Technol.* **43**, 41 (1968).
10. W. R. Lyons and J. S. Dixon, in *The Pituitary Gland*, G. W. Harris and B. T. Donovan, Eds. (Butterworths, London, 1966), vol. 1, p. 527.
11. We thank Miss Eleanor Lasky for technical assistance and the Endocrinology Study Section (NIH) and Dr. Maurice Raben for gifts of ovine prolactin and human GH, respectively. Supported by grants from the PHS (AM 03550 and HD 04063), the University of California School of Medicine, and the Graduate Division of the University of California, San Francisco.

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Habituation and Dishabituation in the Absence of a Central Nervous System

Abstract. *Habituation and dishabituation have been observed in a semi-intact* Aplysia *preparation in which the central nervous system is removed. The amplitude of withdrawal responses in the gill decreases in proportion to the rate of water drops applied (one drop per 0.5 minute to one drop per 2.5 minutes at 15°C). The effects of habituation last for at least 2 hours. A dishabituated response is elicited by stopping the water drops or electrically stimulating the preparation. Furthermore, the gill contains nerve cell bodies, and habituation and dishabituation appear to be properties of these peripheral neurons.*

Habituation has been defined as a progressive reduction in response to repeated stimulation (*1*). The reduction can be reversed (dishabituation) by stopping or changing the stimulus (*2*). It is a process characteristic of the central nervous system of vertebrates and invertebrates (*1*, *3*, *4*) and is described as an elemental form of learning in that an animal has learned not to respond to a particular stimulus (*1*). Habituation also has been reported in semi-intact invertebrate and in spinalized vertebrate preparations (*2*, *5*, *6*). Thus, the intact central nervous system is not necessary for the process to occur. I report that habit-