that this transition when fixed by the measurement of pressure in more suitable apparatus may serve as a very valuable calibration point since the volume change is large and the change in resistance should be easily detectable. Phosphorus has the added advantage that its absorption is low and hence it may be added to samples for x-ray study without impairing the quality of the picture.

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References and Notes

1. P. W. Bridgman, *J. Am. Chem. Soc.* **36**, 1344 (1914).
2. R. W. Keyes, *Phys, Rev.* **92**, 580 (1953).
3. P. W. Bridgman, *Proc. Amer. Acad. Arts Sci.* **76**, 55 (1948).
4. J. C. Jamieson and A. W. Lawson, *J. Appl. Phys.* **33**, 776 (1962); J. C. Jamieson, *Science* **139**, 762 (1963).
5. I thank Dr. Douglas Warschauer for the black phosphorus. Supported by the National Science Foundation grant G19744 and a grant from the Petroleum Research Fund of the American Chemical Society.
6. R. Hultgren, N. S. Gingrich, B. E. Warren, *J. Chem. Phys.* **3**, 351 (1935).
7. P. W. Bridgman, *Proc. Am. Acad. Arts Sci.* **76**, 1 (1945).
8. E. Parthé, *Z. Kristallog.* **115**, 52 (1961).

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Antigenic Determinants in Fragments of Gamma Globulin from Rabbit Serum

Abstract. *Peptic digestion of fraction III from papain-digested rabbit serum γ-globulin produces a variety of smaller fragments. Some are too large to pass through a dialysis bag, and these retain the capacity to precipitate with antiserum to rabbit γ-globulin. Others pass through the bag and fail to precipitate with antibody, but they can inhibit the precipitation of antibody with fraction III. This indicates that antigenic determinants of the γ-globulin molecule are carried in these fragments.*

Rabbit γ-globulin, when digested by crystalline papain, is split into three fragments; each comprises roughly one-third of the parent molecule (*1*). One of these pieces (fraction III) carries a number of properties characteristic of γ-globulin including most of the antigenic determinants (*1*), the ability to fix complement (*2*), reactivity with sera from rheumatoid arthritis patients (*3*), and the capacity to be recognized as γ-globulin by homologous cells (*4*).

Pepsin also splits rabbit γ-globulin into smaller units (*5*), but apparently produces rapid degradation of the portion of the molecule corresponding to fraction III. Therefore, smaller fragments of fraction III possessing these activities might be obtained by controlled treatment with pepsin.

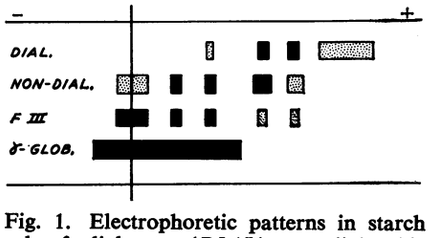

Fig. 1. Electrophoretic patterns in starch gel of dialysate (*DIAL*), nondialyzable fraction (*NON-DIAL*), fraction III (*FIII*), and rabbit γ-globulin (*γ-GLOB*). The solid areas indicate heavy protein concentrations; the speckled areas indicate lower concentrations.

The γ-globulin, prepared from rabbit serum by precipitation with sodium sulfate (*6*), was digested by crystalline papain and fractionated (*1*). Fraction III was digested by 3 percent crystalline pepsin at room temperature, *p*H 4.0, in a dialysis bag under air pressure (2 lb/in.^{2}) within a vessel containing water at *p*H 4.0. The dialysate was changed twice daily over a period of 4 days; by that time the volume within the bag had been greatly reduced and a crystalline precipitate had formed. Both the dialysate and the nondialyzable material were adjusted to *p*H 7.5, and the dialysate was lyophilized.

Starch-gel electrophoresis (*7*) in borate buffer, 0.2*M*, *p*H 8.4, of dialysate, nondialyzable material, rabbit γ-globulin, and fraction III revealed four peaks each for fraction III (F III) and for the nondialyzable material (Fig. 1). These differed quantitatively in that fraction III contains few of the faster moving components, and a large portion of it remained at the origin. The dialysate was composed exclusively of faster moving fractions, much of which appeared as a diffuse smear near the anode end of the gel.

The four components of the nondialyzable fraction were isolated by preparative starch-gel electrophoresis. They were eluted from the starch by electrodialysis. Each was examined in a Spinco model E ultracentrifuge; the sedimentation constant, S_{20}, values ranged from 2.3 to 3.2 as compared to 3.5 for fraction III. Ultracentrifugation of the dialysate gave a slow diffuse boundary that closely resembled a salt boundary.

The nondialyzable fraction gave four precipitin bands in reaction with a goat antiserum to the γ-globulin of rabbit serum, when studied by the Ouchterlony gel-diffusion technique (*8, 9*) (Fig. 2). The specificity of this antiserum was characterized by quantitative precipitin and gel-diffusion tests (*10*). Fraction III gave a single precipitin band, while the dialysate produced no discernible reaction. When the components of the nondialyzable fraction were tested individually, each showed a single precipitin line which gave reactions of identity with bands produced by the others. This suggests that these fragments all carried the same antigenic determinants. The presence of multiple bands in diffusion tests of the whole nondialyzable fraction may have been the result of differences in relative concentration of the components.

Immunoelectrophoresis in agar (*11*) of the nondialyzable material at *p*H 8.6 produced five precipitin lines when developed with the goat antiserum while fraction III itself gave a single band (Fig. 3). Each of the four components of the nondialyzable fraction gave a single band with a characteristic electrophoretic mobility.

The dialysate was chromatographed

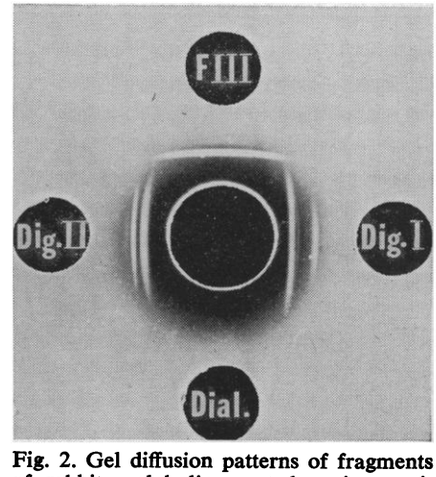

Fig. 2. Gel diffusion patterns of fragments of rabbit γ-globulin reacted against anti-rabbit γ-globulin. Goat antiserum is in central well. Fraction III (*FIII*), nondialyzable fractions of two independent digestions (*Dig. I* and *Dig. II*), and the dialysate from one of the digestions (*Dial.*) are in the peripheral wells.

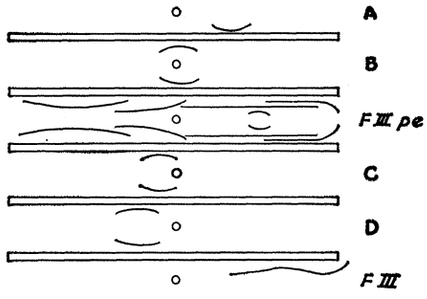

Fig. 3. Immunoelectrophoretic patterns of fraction III (*FIII*) and its digestion products. *FIIIpe,* the nondialyzable material from peptic digestion of fraction III; *A, B, C, D,* the four protein bands from starch-gel electrophoresis of *FIIIpe,* in sequence with *A* the slowest moving and *D* the fastest. The troughs contain goat anti-rabbit γ-globulin serum.

on Sephadex G-50, and two peaks were obtained by elution with 0.01 *M* NaCl. Neither produced specific precipitation with the goat antiserum either in agar (immunoelectrophoresis) or in saline. Both, however, inhibited the precipitation of this antiserum with fraction III, as determined by delay of flocculation time; this demonstrated that the fractions combined with antibody. Neither produced detectable inhibition of the specific precipitin reaction when bovine γ-globulin was added to homologous rabbit antiserum.

It seems likely, in view of the pattern given by starch-gel electrophoresis, that the dialysate consists of a more complex mixture of peptides than the recovery of two peaks from chromatography on Sephadex G-50 would indicate (*12*).

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References and Notes

1. R. R. Porter, *Biochem. J.* **73**, 119 (1959).
2. K. Ishizaka, T. Ishizaka, T. Sugahara, *J. Immunol.* **88**, 690 (1962).
3. J. W. Goodman, *Proc. Soc. Exptl. Biol. Med.* **106**, 822 (1961).
4. F. W. R. Brambell, W. A. Hemmings, C. L. Oakley, R. R. Porter, *Proc. Roy. Soc. London Ser. B* **151**, 478 (1960).
5. A. Nisonoff, F. C. Wissler, L. N. Lipman, D. L. Woernley, *Arch. Biochem. Biophys.* **89**, 230 (1960).
6. R. A. Kekwick, *Biochem. J.* **34**, 1248 (1940).
7. O. Smithies, *ibid.* **61**, 629 (1955).
8. The goat antiserum was provided by Dr. Melvin Cohn.
9. O. Ouchterlony, *Acta Pathol. Microbiol. Scand.* **32**, 231 (1953).
10. J. W. Goodman, *Federation Proc.* **21**, 32 (1962); J. W. Goodman and D. Gross, in preparation.
11. P. Grabar and C. A. Williams, *Biochim. Biophys. Acta* **17**, 67 (1955).
12. Supported by grant G-15604 from the National Science Foundation.

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Escape and Avoidance Learning in Newly Hatched Domestic Chicks

Abstract. *Under the conditions specified, chicks fail to learn either to escape or to avoid shock on the day of hatching. Chicks trained for the first time on the day after hatching quickly learn to escape but do not learn to avoid shock. Avoidance learning first appears on the third day of life, and from that time the number of chicks learning to avoid increases with age, so that by the fifth day of life the majority are able to do so.*

In the course of some experiments on the effects of noxious stimuli on imprinting (*1*), it was noticed that very young domestic chicks seemed to show no concern as they were being harnessed to an apparatus in which they had received a very powerful electric shock some 12 hours previously. The experiment described here was made in order to obtain some systematic evidence on the development of escape and avoidance learning in these animals.

The apparatus consisted of a two-compartment box of a kind commonly used for classroom demonstrations of instrumental avoidance conditioning in rats. One half of the box was painted white and contained a grid floor to which the scrambled shock source (Grason-Stadler model E6070B) supplied a short circuit current of 4 ma at 350 volts, 60 cy a-c. The other half of the box was painted black and had a solid floor. One side of the shock compartment was made of clear Plexiglass through which the conditioned stimulus (CS), the light from a 60-watt lamp placed 4 inches away, could be seen when it was on.

The procedure was as follows: After the chick had been placed in the center of the grid, the CS was turned on. Ten seconds later the shock (UCS) was automatically supplied to the grid in the white compartment, and both CS and UCS remained on for a further 30 seconds or until the chick had crossed to the black half of the box, whichever occurred earlier. The chick was then removed from the apparatus and returned to its cage. There was a 1-minute interval between the start of one trial and the start of the next. Each chick was run for 100 trials, or until it had reached a criterion of five successive avoidances or eight avoidances in ten consecutive trials, whichever occurred first. A thin coating of electrode paste was smeared on the chick's feet every ten trials. Control chicks were run at the same time in a dummy apparatus; they were treated in exactly the same way except that they were never given an electric shock.

Fifty-five New Hampshire × Barred Rock chicks were hatched in the laboratory and reared in individual fiber-glass cages in a constantly illuminated room kept at 90°F. They were divided into five independent groups; seven chicks were trained when they were less than 15 hours old (day 1), ten when they were between 25 and 43 hours old (day 2), nine when they were between 49 and 67 hours old (day 3), nine when they were between 73 and 91 hours old (day 4), and ten when they were between 97 and 115 hours old (day 5). Two additional chicks were run as controls (no shock) at corresponding ages.

The results were as follows: None of the day-1 group learned either to escape or to avoid the shock; all except one of the day-2 group learned to escape but none learned to avoid; from day-3 the proportion of chicks learning to avoid increases with age. The mean escape latencies of the chicks in the different groups which failed to learn to avoid shock are given in Fig. 1, from

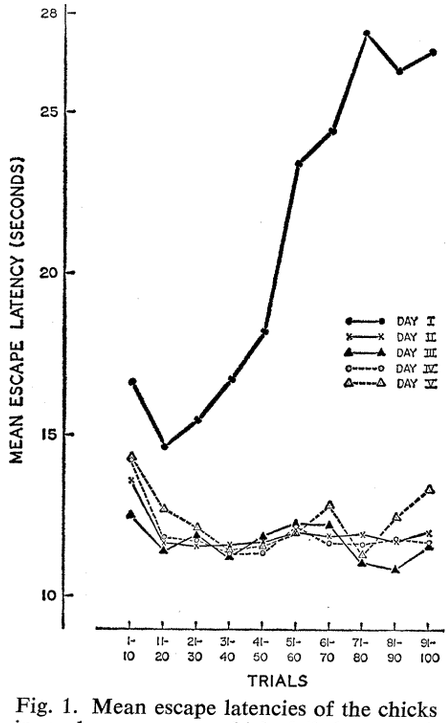

Fig. 1. Mean escape latencies of the chicks in each age group which failed to reach the criterion of avoidance learning.

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