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# Antigenic Properties of Some Azo Compounds of Serum Albumin and Serum Globulin.

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Mulinos and Schlesinger[1] have shown that when the proteins of egg white or of homologous blood serum are linked to antipyrine through an azo group,* the resulting compounds are antigens capable of eliciting precipitins in rabbits and shock or uterine spasm in guinea pigs. The present report relates to similar experiments with analogous antigens prepared by coupling diazo compounds with homologous serum albumin and globulin.

The results with the azo proteins from serum albumin were analogous to those obtained with whole serum, in that precipitative reactions were readily obtained and were inhibited by the preliminary addition of the original amino compounds employed for the preparation of the azo proteins. On the other hand, the azo derivatives of serum globulin failed to display antigenic character.

The serum albumin and globulins, prepared in the usual manner by precipitation from saturated and half-saturated ammonium sulfate solution respectively, were coupled in weakly alkaline solution, according to the procedure of Landsteiner and Lampl,[2] with the diazo compounds prepared from 4-amino antipyrine and anthranilic acid. After 2 hours at low temperature the mixture was acidified with acetic acid. The flocculent protein was repeatedly redissolved, then reprecipitated by acid, until the supernatant liquor remained colorless. The final azo-protein solutions were made up to contain 10 mg. of protein per cc.

The effects of precipitins from rabbits upon the 4 types of azo protein are shown in Table I; inhibition by amino antipyrine and anthranilic acid is shown in Table II. The cases in which the azo groups showed antigenic reactions may be ascribed to the presence of serum albumin as a contaminant in the original globulin. It is of

1 Mulinos and Schlesinger, PROC. SOC. EXP. BIOL. AND MED., 1937, **35**, 305.

* It was stated in the paper by Mulinos and Schlesinger[1] that antipyrine was diazotized and coupled to protein. In the process the intermediary compound, 4-amino antipyrine, was synthesized and employed.

2 Landsteiner and Lampl, *Biochem. Z.*, 1918, **86**, 342.

TABLE I.

Precipitative Reactions with Hapten Coupled to Rabbit's Serum Proteins.

| Dilution of Antigen | 4-amino-antipyrine coupled to: Albumin a | b | c | d | e | 4-amino-antipyrine coupled to: Globulin a | b | c | d | Anthranilic acid coupled to: Albumin a | b | Anthranilic acid coupled to: Globulin a | b |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1:1000 | ± | + | + | ± | + | ± | 0 | 0 | 0 | + | + | ± | + |
| 1:2000 | + | + | + | ± | + | + | 0 | 0 | 0 | + | + | ± | + |
| 1:4000 | + | + | + | ± | + | + | 0 | 0 | 0 | + | + | ± | + |
| 1:8000 | + | + | + | ± | + | + | 0 | 0 | 0 | + | + | ± | + |
| 1:16000 | + | + | + | ± | ± | + | 0 | 0 | 0 | + | + | ± | ± |

interest that the azo globulins were approximately twice as intensely colored as the azo albumins.

TABLE II.
Influence of Specific Non-conjugative Chemicals on Precipitative Reactions.

| | Antigen | | | |
|---|---|---|---|---|
| | A | | B | |
| | Rabbit's serum albumin coupled to: | | As in A, plus pure | |
| Animal No. | 4-amino antipyrine | Anthranilic acid | Antipyrine | Benzoic acid |
| 65 | ++++ | — | ± | — |
| 67 | +++ | — | ± | — |
| 43 | ++± | — | trace | — |
| 7 | + | — | faint trace | — |
| 45 | ++ | — | faint trace | — |
| 41 | +± | — | 0 | — |
| 325 | — | +++ | — | + |
| 347 | — | ++± | — | ± |
| 827 | — | + | — | 0 |
| 432 | — | ++ | — | faint trace |
| 377 | — | ++ | — | " " |

TABLE III
Guinea-pig Uterus: Pigs Injected with Homologous-protein Compounds.

| Guinea pig injected with: | | No. experiments | Specific spasm of uterus | Specific Inhibition |
|---|---|---|---|---|
| Anthranilic acid-azo-guinea-pig serum: | Albumin | 5 | 4 | 3* |
| | Globulin | 5 | none | — |
| 4-amino-antipyrine guinea-pig serum: | Albumin | 6 | 4 | 4† |
| | Globulin | 4 | none | — |

*By the addition of pure sodium benzoate to the buffered Locke's solution.
†By the addition of pure antipyrine to the buffered Locke's solution.

Table III shows the results of experiments with guinea pigs. The animals were injected with the 4 azo compounds produced from amino antipyrine and anthranilic acid with guinea pig's serum albumin and globulin respectively. After the requisite incubationary period the uteri of the guinea pigs were excised and suspended in warm, oxygenated Ringer's solution, according to the technic of Dale.[3] To one strip of uterus was added the homologous azo protein. If spasm occurred, the strip was washed and another dose of the azo protein was added. If this second addition failed to induce spasm, it was assumed that the first spasm was specific in nature. To a second strip of a uterus found sensitive was added a preliminary dose of the chemical compound corresponding to the azo pro-

[3] Dale, *J. Pharm. Exp. Therap.*, 1912, 4, 167.

tein. To a third strip of such a uterus was added another azo protein prepared from a foreign protein (rabbit or egg) and the same amino compound. When a strip of a uterus sensitive to an azo albumin was treated with the simple amino compound, it lost its ability to respond to spasm to the addition of the specific antigen. The strip was therefore rendered insensitive by the amino compounds which were non-antigenic in themselves but had conferred upon the homologous protein antigenic powers for the animal from which it was derived. Of 9 guinea pigs injected with azo globulins derived from anthranilic acid or amino antipyrine, none became sensitive either to the original azo globulin or to the azo albumin. Of 11 animals injected with the azo albumin compounds and studied in parallel with the above, 8 (73%) were positive both to the original azo albumin and also to the azo globulin. Seven of the 8 sensitive uteri showed the phenomenon of "specific inhibition" upon preliminary addition of the corresponding chemical compound; in the eighth uterus the response was present but much diminished. No spasm occurred with the native proteins or with antigens prepared from amino compounds other than those employed in the preparation of the azo protein used for sensitization.

Analogous experiments independently undertaken by one of us (DBS) with azo proteins prepared from diazotized sulfanilic acid and rabbit-serum albumin and globulin yielded similar results. The azo proteins were prepared by the method of Heidelberger.[4] The azo albumin was purified by repeated precipitation with acid; the azo globulin was purified similarly but in presence of ammonium sulfate. The purification was continued until the azo proteins gave no precipitate with chicken anti-rabbit serum. Both preparations were redissolved in neutral solution and dialyzed until no more color diffused out. Rabbits were injected with 2.5 mg. of protein 5 times a week for 2 weeks, and 5 mg. of protein 5 times a week for a third week. They were bled on the fifth day after the last injection. Of the 10 rabbits, the 5 injected with the azo globulin were negative, and 3 of the 5 injected with azo albumin were positive. This result was unchanged by a second course of injections. The 3 positive sera gave weak cross-reactions with azo-egg-albumin prepared from diazotized sulfanilic acid. After a third course of injections all of the rabbits injected with azo-albumin compounds gave positive results, while those injected with the azo globulin still gave negative results. Six more rabbits given 2 more courses of injections of azo globulin again gave negative results. The precipitates obtained in

[4] Heidelberger, Kendall, and Soo Hoo, *J. Exp. Med.*, 1933, **58**, 137.

the azo-albumin antiazo-albumin reaction were found colorimetrically to contain only about 10% of the colored antigen. In a cross-reaction between azo globulin and antiazo-albumin the precipitate contained less than 4% of the colored component, despite the fact that the azo-globulin solutions are colored more intensely per unit of nitrogenous substance in solution.

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## Carrion's Disease. I. Behavior of the Etiological Agent Within Cells Growing or Surviving *in vitro*.*

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"Carrion's Disease" commonly includes two very different clinical syndromes—(1) a severe, usually fatal febrile anemia, (Oroya fever) and (2) a remarkable cutaneous eruption of hemangioma-like nodules *(verruga peruana)*. Clinical and experimental evidence strongly suggests that these two conditions are successive stages of a single disease, the first (anemic) stage being of variable severity and often inapparent or even absent.

The generally accepted cause of the first condition (Oroya fever) is a small pleomorphic microörganism, first seen by Barton[1] in or on red blood cells and named *Bartonella bacilliformis*.[2] This organism was grown in Noguchi's leptospira-medium.[3] Apparently iden-

* This paper, with the four following papers, summarizes certain observations made by the Harvard 1937 Expedition to Peru, organized by Dr. Richard P. Strong of the Department of Tropical Medicine. Other members were Dr. Henry Pinkerton, Department of Pathology, Dr. Marshall Hertig and Dr. David Weinman, Department of Comparative Pathology, and Mr. Byron L. Bennett, Technical Assistant. The work was made possible by the generous coöperation of Dr. Telémaco S. Battistini, Director of the National Institute of Hygiene and Public Health in Lima, and was carried out in this Institute. Assistance rendered by other Peruvian physicians is also gratefully acknowledged. These investigations were aided in part through grants from the Richard P. Strong Fund (founded by the late Dr. Frederick C. Shattuck), the Proctor Fund for the Study of Chronic Disease, and the William W. Wellington Memorial Research Fund.

1 Barton, A. L., *Cron. Med. Lima*, 1909, **26**, 7.

2 Strong, Tyzzer, Brues, Sellards, and Gastiaburú, *Report of First Expedition to South America*, Harvard University Press, 1915.

3 Noguchi, H., and Battistini, T. S., *J. Exp. Med.*, 1926, **43**, 851.