On the Failure of Azo-gelatin as an Antigen

By F. HAUROWITZ, M. TUNCA AND PAULA SCHWERIN, *From the Institute of Biological and Medical Chemistry, University of Istanbul*

(Received 2 January 1943)

The non-antigenicity of gelatin was attributed for a long time to its lack of aromatic amino-acids. Later, Landsteiner [1919] and Hooker & Boyd [1932] introduced aromatic determinant groups into the gelatin molecule by coupling it with diazotized arsanilic acid. Injection of arsanil-azo-gelatin into rabbits furnished immune sera which gave slight precipitates with the arsanil-azo-compounds of casein, ovalbumin and serum-globulin, but gave no visible reaction with arsanil-azo-gelatin. Similar results were obtained by coupling gelatin with glucosido-tyrosine [Clutton, Harington & Yuill, 1938]. These experiments demonstrated clearly that the absence of antigenicity in gelatin cannot be attributed merely to the deficiency of aromatic groups.

In previous papers [Haurowitz, 1936; Haurowitz & Kraus, 1936] we showed that the bulk of intravenously injected arsanil-azo-globulin is fixed in the liver and the bone-marrow of rabbits. We have now performed similar experiments with arsanil-azo-gelatin.

METHODS

Arsanil-azo-globulin was prepared in the usual way by coupling pseudo-globulin from sheep serum with diazotized arsanilic acid [Haurowitz, 1936]. For arsanil-azo-gelatin 1.35 g. of arsanilic acid were diazotized in the usual manner

Haurowitz & Kraus, 1936; Haurowitz & Breinl, 1932]. The animals were killed by bleeding 60 min. after the injection of the azo-protein.

RESULTS

Deposition of arsenic in the liver. As will be noted from Table 1 only 4.4% of the injected arsenic was deposited in the liver, whereas 66% passed into the urine. In a second rabbit, given an injection of arsanil-azo-globulin under similar conditions, 34% of the injected arsenic was found in the liver and only 9% passed into the urine. A repetition of this experiment on two other animals furnished analogous results.

Excretion of arsenic. The experiments recorded in Table 2 were performed on rabbits weighing 1.7–2.2 kg. In each experiment a pair of animals was used, since the food varied somewhat from day to day, both animals of a pair receiving the same food. One animal received an intravenous injection of the arsanil-azo-gelatin, the other animal received arsanil-azo-globulin as a control. The animals were kept in metabolism cages and the urines collected quantitatively during 2 days. Arsenic was determined in 10 or in 20 ml. of urine according to our previously described method.

Table 1. *Distribution of arsenic 60 min. after the intravenous injection of arsanil-azo-gelatin and arsanil-azo-globulin into rabbits*

Injected protein	50 mg. of arsanil-azo-gelatin (=1.8 mg. As)	60 mg. of arsanil-azo-sheep serum globulin (=0.96 mg. As)
Wt. of rabbit	2200 g.	1760 g.
Liver: (a) Weight	67 g.	57.5 g.
(b) Content of As	0.08 mg. (=4.4% of injected As)	0.33 mg. (=34% of injected As)
Bone marrow	0.25 mg. As/100 g.	0.71 mg. As/100 g.
Blood	0.025 mg. As/100 ml.	0.30 mg. As/100 ml.
Kidneys	0.075 mg. As	0.072 mg. As
Urine: (a) Volume	9.5 ml.	7.0 ml.
(b) Content of As	1.2 mg. (=66% of injected As)	0.088 mg. (=9.2% of injected As)

[Haurowitz, 1936], and the solution added to a chilled solution of 4.5 g. of gelatin in 140 ml. of 0.65 *N* NaOH. The mixture was permitted to stand at 0° for 16 hr. and then neutralized, precipitated by twice its volume of acetone, dissolved in water and dialysed for 48 hr. against distilled water. The solutions for injection into rabbits contained 2–3% of azo-protein. The intravenous injection into rabbits and the determination of arsenic in the organs have been described in previous papers [Haurowitz, 1936;

In each of these experiments arsenic was eliminated more rapidly by the animals which had been injected with arsanil-azo-gelatin (Table 2).

The rapid elimination of arsenic after the injection of arsanil-azo-gelatin cannot be attributed to the presence of dialysable compounds of arsenic in the injected preparation, for it had been purified by dialysis, and further dialysis in distilled water

Table 2. *Excretion of arsenic after the injection of arsanil-azo-gelatin and arsanil-azo-globulin*

Exp. no.	Rabbit no.	Injected arsanil-azo-protein		Content of As mg.	Content of arsenic in urine	
		Wt. mg.	Protein		24 hr. mg.	48 hr. mg.
1	208	60	Globulin	2.5	0.91	—
	209a	50	Gelatin	1.8	1.54	—
2	210	60	Globulin	2.5	0.88	—
	209b	50	Gelatin	1.8	1.71	—
3	213	48	Globulin	2.0	0.28	0.06
	214	45	Gelatin	1.6	0.99	0.05
4	215	48	Globulin	2.0	0.47	0.02
	217	56	Gelatin	2.0	0.75	0.07

yielded only negligible quantities of dialysable As compounds.

The rapid elimination of As when administered intravenously as arsanil-azo-gelatin appears to be related to the peculiarities of the gelatin molecule,

e.g. its strong tendency to remain in solution and not to be adsorbed. It is assumed therefore, that the failure of arsanil-azo-gelatin to produce antibodies is chiefly due to the fact that its molecules are not deposited at the sites of antibody-formation, but are rapidly eliminated from the organism. Probably this is also the reason for the lack of antigenicity of pure gelatin.

SUMMARY

1. While an intravenous injection of arsanil-azo-globulin into rabbits gives rise to a considerable deposition of arsenic in the liver, an analogous injection of arsanil-azo-gelatin is followed by rapid urinary excretion of arsenic, and only small quantities of arsenic are deposited in the liver.

2. The failure of arsanil-azo-gelatin to act as full antigen is attributed to the insufficient deposition of this substance in the reticulo-endothelial cells, i.e. in the cells producing the antibodies.

REFERENCES

- Clutton, R. F., Harington, C. R. & Yuill, M. E. [1938]. *Biochem. J.* **32**, 1111.
 Haurowitz, F. [1936]. *Hoppe-Seyl. Z.* **245**, 23.
 — & Breinl, F. [1932]. *Hoppe-Seyl. Z.* **205**, 259.
 Haurowitz, F. & Kraus, F. [1936]. *Hoppe-Seyl. Z.* **239**, 76.
 Hooker, S. B. & Boyd, W. C. [1932]. *J. Immunol.* **24**, 141.
 Landsteiner, K. [1919]. *Biochem. Z.* **93**, 106.

Riboflavin and Riboflavin Adenine Dinucleotide in Ox Ocular Tissues

By FLORA J. PHILPOT AND ANTOINETTE PIRIE, *From the Oxford Nutrition Survey and the Nuffield Laboratory of Ophthalmology, Oxford Eye Hospital*

(Received 2 January 1943)

Sherman & Sandel [1931] found that vitamin 'G'-deficient rats showed eye changes which they described as 'a very slight keratitis with corneal corpuscles, a few polymorphs and some new formed blood channels in the cornea'. Day, Langston & O'Brien [1931] also observed that rats on a 'G'-deficient diet developed eye lesions. Their animals not only developed a condition which they described as anterior interstitial keratitis, but also cataract. The lens changes in vitamin 'G' deficiency were further investigated by many different workers, but the original observations could not be repeated. The changes in the cornea were not examined in detail until Bessey & Wolbach [1939] and El Sadr [1939] published descriptions of the vascularization of rat corneas in riboflavin deficiency. In the meantime vitamin 'G' had become identified with vitamin B₂ or riboflavin. Sydenstricker, Sebrell, Cleckley & Kruse [1940] described vascularization of the cornea in man in riboflavin

deficiency. Patek, Post & Victor [1941] have found that it occurs in pigs, and Street, Cowgill & Zimmerman [1941] have described its occurrence in dogs.

Apart from estimations of the total flavin content of the retinae of different species by v. Euler & Adler [1933; 1934a] no determinations of the amount or type of flavin in the different parts of the eye have been published. We have now estimated total flavin and riboflavin adenine dinucleotide in ox eyes in order to see whether a determination of the distribution and type of flavin present would help to explain the sensitivity of the cornea to riboflavin deficiency.

METHODS

(1) Collection of material

Ox eyes were removed and put into a refrigerator within 5 min. of the death of the animal. As soon