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Effect on Antibody Production of Injection of an α_2 -Protein Fraction

WHILE investigating the effect of large doses of fractions of blood on the survival of rat skin homografts, it was found that intraperitoneal injections of an α_2 -protein fraction from human, rat, rabbit, or bovine sera could prolong the survival of homografts in outbred albino rats. At the time of writing, 4 of 20 of these grafts have survived for eight and 4 of a further 20 for six months and are still apparently healthy. This fraction was prepared from whole serum by a modification of the chromatographic method of Steinbuch and Quentin¹ on DEAE cellulose. Of Steinbuch and Quentin's three fractions only that eluted with 0.5 molar pH 5 acetate buffer was effective. Kamrin² had previously produced a marked prolongation of survival of rat skin homografts by injecting a series of plasma fractions, of which the most potent contained the highest concentration of α_2 -protein. It seems, therefore, that the fractions used by Kamrin and the fraction I have used probably contain a common active principle.

The work with the 0.5 molar eluate has also shown that this fraction inhibits the production of antibodies to other antigens.

The immune elimination technique of Talmage *et al.*³ was used as an index of antibody production. 10 mg of human albumen labelled with iodine-131 was injected into six rabbits each of which had received 100 mg of the eluate intraperitoneally 30 min after being given the antigen and again three days later. There was no evidence of immune elimination in five of the six rabbits, but in the remaining rabbit immune elimination was observed on the eighth day. Nine rabbits injected only with the labelled human albumen all showed immune elimination between the eighth and eleventh days ($0.01 > P > 0.001$).

In rabbits injected intravenously with 1.0 ml. of packed, washed human red cells haemagglutinating antibody developed in each of nine control animals, but it could be detected in only two of ten animals treated by intraperitoneal injection of 100 mg of the eluate given 2 h before injection of the red cells.

The timing of the injection of the protein fraction was found to be important. Suppression of immune elimination of iodine-131-labelled human albumen was observed in all animals receiving the fraction 1 or 2 h before administration of the antigen. Normal immune elimination was observed in all animals receiving the fraction 4 h after the antigen. The production of haemagglutinating antibody was unaffected if the fraction was injected later than 15 min before the antigen, and was most effective if given 2 h before.

It therefore seems likely that the protein fraction affects the early stages of the immune response.

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PATHOLOGY

Platelet Clumping *in vivo*

Wharton-Jones¹ and Bizzozero² showed that, when an artery was injured, white masses would build up at the site of injury and embolize. Honour and Ross-Russell³ examined this response to injury in considerable detail, in arteries on the surface of the rabbit brain. They showed that the white bodies were composed of closely packed but morphologically discrete platelets, and they were unable to influence the rate of formation of the white bodies or their size by heparin, anti-histamines, or 5-hydroxytryptamine (5-HT) antagonists. The demonstration by Gaarder *et al.*⁴ that adenosine diphosphate (ADP) was capable of producing clumping of human platelets *in vitro*, led Mitchell and Sharp⁵ to investigate related substances, together with other substances normally carried by the platelet. They confirmed that ADP clumped citrated human platelets at a concentration of 0.04 $\mu\text{g}/\text{ml}$. and that adenosine triphosphate (ATP) was much less active, a concentration of 1.5 $\mu\text{g}/\text{ml}$. being required. They also found that human platelets were clumped by 5-HT, and by noradrenaline at a concentration of 0.06 $\mu\text{g}/\text{ml}$. Rabbit platelets were clumped by ADP at a concentration of 0.05 $\mu\text{g}/\text{ml}$., were much more sensitive to ATP than human platelets, 0.22 $\mu\text{g}/\text{ml}$. sufficing to produce clumping, were insensitive to noradrenaline, and showed a variable response to 5-HT.

These observations led us to examine the effect of ADP, ATP and 5-HT on injured brain vessels in the rabbit.

(1) *The major injury.* The cerebral cortical arteries of anaesthetized rabbits were exposed and observed microscopically using the techniques described by Honour and Ross-Russell³. An artery 100–200 μ in diameter was then pinched firmly with needle-pointed ophthalmic forceps, producing slight external bleeding, and transient vaso-constriction. When this subsided a steady succession of white bodies would form at the site of injury and embolize. The application of ADP, ATP and 5-HT solutions to the external surface of such a site did not modify the size or rate of production of the white bodies which were forming spontaneously.

(2) *The minor injury.* If, however, a cortical artery was gripped gently with the forceps, no external bleeding occurred and apart from transient vaso-constriction the site looked normal. No white bodies formed spontaneously, but the application to such a site of ADP, ATP or 5-HT dissolved in 0.9 per cent saline produced white bodies, which continued to build up and embolize so long as the active agent was applied, production stopping as soon as the application was discontinued. The lowest concentration of these agents which would produce white bodies when applied to the outside of a minimally injured rabbit brain artery was determined, each substance being tested on 8 rabbits. The results were:

Substance	Range of lowest effective conc. ($\mu\text{g}/\text{ml}$.)	Mean ($\mu\text{g}/\text{ml}$.)
ADP	0.25–16.0	3.0
ATP	0.25–16.0	5.3
5-HT	0.125–32.0	4.8

Application of the test solutions to uninjured cortical arteries did not produce white bodies.

(3) *Effect of 5-HT antagonists.* Minor injuries were inflicted on cortical arteries in two rabbits and their