Alloxan Diabetes and Kidney Function

IT is a well-known fact that the intravenous injection of high diabetogenic doses of alloxan (80–100 mgm. per kgm.) in the dog produces a very severe diabetic-uræmic syndrome. With such doses the death of the animals follows as a rule within one week, the cause of the death being probably due to the disturbance of the renal function[1,2,3]. In the course of our experiments on alloxan diabetes in the dog, we have been faced with this fact, which prevented us from keeping the animals with severe diabetes for further study. It was thought that clamping of the renal vessels previous to the alloxan injection, maintained a few minutes after the end of the injection, would avoid the kidney damage, since we have been able to demonstrate the rapid inactivation of the alloxan in contact with the blood and body tissues[4]. Our former experience shows, in fact that after ten minutes of contact with blood at 37° C. *in vitro* a diabetogenic dose of 100 mgm. alloxan per kgm. does not evoke its diabetogenic effect.

In order to test our theory the following experiments were performed: a group of five normal dogs were injected with alloxan during clamping of the renal vessels. Two of the dogs received 80 mgm. of the drug per kgm., and the other three 100 mgm. per kgm. Just before the alloxan injection in the saphenous vein, the abdomen was opened under local anæsthesia (with procaine solution, without adrenaline), and the usual aseptic care. After dissection of the renal pedicles, one clamp was placed in each side, suppressing the blood flow in both kidneys. The alloxan was then injected, and the clamps removed ten minutes after the end of the injection. The abdomen was closed with suture, and the animal, which behaves as a normal one, is replaced in the cage. Venous blood samples are taken for glucose and urea estimations, just before the injection of alloxan, and afterwards every hour for eight or ten hours, and on the following days.

Other five dogs have been treated in the same way (including procaine, opening of the abdomen, suture, etc.) but no clamps were placed on the kidney vessels.

TABLE 1. EFFECT OF INTRAVENOUS INJECTION OF ALLOXAN IN THE DOG

(*a*) Dogs with clamped kidney vessels.

Dog number	Alloxan mgm./kgm.	Blood sugar (mgm. per 100 c.c.) Before alloxan	After alloxan (hours) 1	2	3	4	5	6	7	8	24	48
248	90	80	—	73	67	40	23	23	20	23	117	117
249	90	87	90	127	103	87	70	50	47	27	77	60
250	100	80	90	153	132	80	43	50	43	50	153	103
251	100	97	173	160	137	10[illegible]	177	33	27	37	130	103
252	100	93	170	163	107	93	87	80	60	50	93	88

(*b*) Dogs with non-clamped kidney vessels

240	80	77	143	177	197	207	143	83	43	33	320	1060
253	100	73	170	167	143	110	77	37	70	27	70	1000
254	100	90	140	—	—	—	—	—	—	—	237	347
262	100	77	147	200	—	—	—	—	—	—	280	—
263	100	87	163	190	—	—	—	—	—	—	197	657

TABLE 2. BLOOD UREA IN DOGS AFTER ALLOXAN INJECTION. DOGS FROM TABLE 1. UREA IN MGM. PER 100 C.C.

Clamped kidney vessels				Unclamped kidney vessels			
Dog number	Before alloxan	Hours after alloxan 24	48	Dog number	Before alloxan	Hours after alloxan 24	48
248	42	80	42	240	56	480	688
249	32	32	52	253	28	112	360
250	52	64	66	284	—	240	544
251	40	38	60	262	40	152	—
252	36	44	62	263	40	140	512

As seen in Table 1, both groups of dogs show the known glycæmic response to the alloxan, but, surprisingly, the dogs with clamped kidney vessels do not have hyperglycæmia forty-eight hours after the injection. These dogs are neither diabetic nor uræmic, and in contrast with the non-clamped ones they live without hyperglycæmia, glycosuria or elevation of blood urea, and with a normal aspect, two months after the administration of alloxan. The unclamped dogs died between two and seven days after the injection with hyperglycæmia and very high uræmia (Table 2).

It seems, therefore, that avoiding the contact between the kidneys and the blood carrying alloxan, during the time necessary for the inactivation of the drug, not only prevents the kidney damage and the uræmia, but also the diabetic disturbance. These results indicate that the kidney plays some hitherto unknown part in the development of alloxan diabetes; the contact between alloxan and the kidney is apparently necessary for the display of the full diabetogenic effect.

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An 'Incomplete' Form of α Agglutinin

IN the *Rh* system of blood groups two forms of antibody have been described, an agglutinin and an 'incomplete', 'blocking' or 'conglutinating' antibody[1,2]. The iso-agglutinin can be detected by the ordinary iso-agglutinin technique[3], which, however, fails to detect the incomplete antibody. The presence of the latter in a serum can, however, be demonstrated by the blocking test[1], the Coombs test[4], the Diamond slide test[5], the conglutination test[6], and the albumen test[7].

Attempts to demonstrate an incomplete antibody in the *ABO* system have heretofore proved unsuccessful. However, the fact that with certain anti-*A* sera better agglutination with group A_1 red cells was obtained at a dilution of 1:16 or 1:32 than with undiluted serum[8] seemed to us to indicate the possible presence of an 'incomplete' or 'blocking' antibody. Two such sera, therefore, were chosen and tested.

These were very potent immune anti-*A* sera from persons of group *O* (Taylor–Sparks) produced as a result of injection with *A* group specific substance isolated from pseudomucinous cyst[9]. It was thus first necessary to inactivate the iso-agglutinin, which was readily detectable at all dilutions up to a titre of 16,000 and 8,000 respectively. It has been shown[10] that while the anti-*Rh* agglutinin is rendered inactive by heating at 70° C. for 5–10 minutes, the incomplete antibody is still active. However, as the anti-*A* agglutinin seems to be more heat-stable than the anti-*Rh*, the sera containing immune anti-*A* agglutinins were heated for 20 minutes at approximately 75° C., after which they were tested against A_1 cells at room temperature and were found to give no agglutination. With A_2 cells there was slight agglutination ((+)); with *B* cells the agglutination was slightly stronger.

The heated sera were then tested for the possible presence of an incomplete form of anti-*A* antibody by the blocking test[1]. One volume of serum and one volume of a 2 per cent suspension of A_1 red cells were mixed in a small tube and allowed to stand at room temperature for one hour. The supernatant fluid was then withdrawn from the tube and a unit volume of a strongly agglutinating anti-*A* grouping serum (titre 512) was added. A control tube, containing the same A_1 red cells, which, however, had not been exposed to the test sera (Taylor–Sparks), and a volume of the anti-*A* grouping serum, was included in the experiment. After two hours at room temperature, the A_1 red cells which had first been treated with the heated test sera (Taylor and Sparks) gave no agglutination with the anti-*A* serum, whereas in the control tube the red cells were completely agglutinated. This experiment clearly demonstrated that the expected agglutination between the *A* cells and the anti-*A* serum had been blocked by a factor contained in the sera (Taylor and Sparks), whereas the red cells which were not first exposed to the Taylor and Sparks sera were agglutinated normally. To show that the blocking was specific for the *A* cells, blocking tests were also carried out using group *B* cells. In these there was no blocking of the anti-*B* agglutination.

It was next decided to attempt to demonstrate the presence of the blocking antibody in the sera (Taylor and Sparks) by the Coombs test. One volume of each of the heated sera was mixed with a 2 per cent suspension of A_1 red cells and allowed to stand for 1 hour at room temperature as for the blocking test. The A_1 red cells were then carefully washed and an anti-human serum rabbit serum added to them. Almost immediately the cells were strongly clumped, showing that they had become sensitized by an antibody. Group *O* cells subjected to the same treatment were not clumped.

The blocking or incomplete antibody in Taylor and Sparks heated sera was also demonstrated well by the Diamond slide test, and rather less conclusively by the Diamond albumen test.

It may be significant that in both the instances the incomplete *A* antibody was found in an immune serum (it is possible that it does not occur naturally and is only produced as a result of stimulation by the homologous antigen). We are planning further work to elucidate this point, but our experiments to date have shown that incomplete *A* antibodies occur and, furthermore, that a positive Coombs reaction can be obtained in the *ABO* system.

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Enhancement of Immune Antibodies by Human Serum

IT has been observed that the use of human serum, instead of saline, as a diluent in titration of immune agglutinins (*A*, *B*, *Rh*) enhances the action of these antibodies, and higher titres are therefore obtained[1]. Similarly, the 'conglutination-test' for the detection of *Rh* sensitization is also based on the use of human serum, instead of saline, for dilution in titration[2]. In describing the 'conglutination-reaction', Wiener suggested that this is due to a serum factor, a protein, which is not fully developed in the fœtus and is formed only shortly after delivery[2,3]. The post-natal formation of sufficient quantities of this protein would presumably account for the development of erythroblastosis fœtalis after delivery, and not during pregnancy.

We have tried to determine whether the property of serum to enhance the action of immune antibodies is present in sera of new-