Summary. Spectrophotometric determination of RNA and isotopic determination of the incorporation of uridine 5'-monophosphate H^3 into RNA have shown that aldosterone stimulates RNA-synthesis *in vivo*. This occurs during the time aldosterone exerts its antinatruretic action. Actinomycin D, an inhibitor of RNA-synthesis, blocked both of these effects. These observations are consistent with the hypothesis that the antinatruretic action of aldosterone is mediated *via* DNA directed synthesis of messenger-RNA which in turn stimulates the synthesis of a protein involved in the transport of sodium.

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Effect of Bovine Gamma Globulin on Subsequent Immunization with Dinitrophenylated Bovine Gamma Globulin.* (30165)

HENRY ASHLEY AND ZOLTAN OVARY†
Department of Pathology, New York University School of Medicine, New York City

It has been shown that when rabbits are immunized with a hapten-protein conjugate, an anamnestic response can be obtained only when the same hapten-carrier protein conjugate is used for reinjection. The same hapten on a different, unrelated carrier protein does not provoke an anamnestic response(1).

The present study reports results obtained when the carrier protein is injected prior to injection of the hapten-protein conjugate.

Materials and methods. Animals. Random-bred, albino rabbits weighing approximately 3-4 kg were used for immunization. Hartley-strain, albino guinea pigs 250 ± 50 g, were used for passive cutaneous anaphylaxis (PCA) reactions.

Antigens. (a) Bovine gamma globulin (BGG) Armour Pharmaceuticals, Lot T 30203. (b) 2,4-dinitrophenylsulphonic acid (DNPS) (Eastman Kodak, Rochester, N. Y.) twice recrystallized from water and used for coupling to 2 different carrier proteins, *i.e.*, BGG and crystalline bovine serum albumin (BSA) (Armour Pharmaceuticals, Lot W 69312).

Coupling. Coupling was effected as described previously(1). Four different preparations of dinitrophenylated BGG (DNP-BGG) were used for immunization. These preparations differed only in the numbers of dinitrophenyl (DNP) groups present per molecule of BGG. The numbers of DNP groups per molecule (G/M) were 15, 33, 47, and 55.

The dinitrophenylated bovine serum albumin (DNP-BSA) had 28 G/M and was used for eliciting PCA reactions only.

Immunization. Two experiments were performed, with 20 rabbits in each. Ten rabbits were pre-immunized with BGG, and 10 were used as controls in each experiment. The pre-immunized rabbits received 3 mg of BGG in saline for 3 consecutive days. Sixteen days

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TABLE I. PCA Reactions Obtained with 500 μg DNP-BSA as Challenging Antigen per Guinea Pig.

Immunization of rabbits	Titer of sera: Days after last injection of DNP-BGG 3	5	7	9	11	14	17
BGG then DNP-BGG 15 G/M 4 rabbits (one died)	0	0	0	0	0	nd	0
BGG then DNP-BGG 33 G/M 5 rabbits	0	0	0	0	0	nd	0
BGG then DNP-BGG 47 G/M 5 rabbits	0	0	0	0	0	nd	0
BGG then DNP-BGG 55 G/M 5 rabbits	0	0	0	0	0	nd	0
DNP-BGG 15 G/M 5 rabbits	0	0	0	0	0	nd	0
DNP-BGG 33 G/M 5 rabbits	0	0	0	0	0	nd	0
DNP-BGG 47 G/M 5 rabbits	0	0	0	0	0	nd	0
	0	0	1/10	1/50	1/50	nd	1/10
	0	0	1/25	1/50	1/50	nd	0
	0	0	1/25	1/50	1/50	nd	0
	0	0	1/25	1/50	1/50	nd	0
DNP-BGG 55 G/M 4 rabbits	0	0	0	1/25	1/25	nd	0
	0	0	1/25	1/25	1/50	nd	1/25
	0	0	1/25	1/50	1/50	nd	1/25
	0	0	1/25	1/50	1/50	nd	1/25

See remarks on Table II.

after the first injection, the pre-immunized animals were divided into 2 groups of 5 animals. In the first experiment, 5 pre-immunized animals and 5 controls received 3 mg of DNP-BGG, 33 G/M, in saline intravenously for 3 consecutive days. The remaining 5 of each category were treated similarly, except that a preparation of DNP-BGG with 47 G/M was used.

In the second experiment, the only difference was that the preparations of DNP-BGG differed in the number of G/M. One preparation had 15 G/M, whereas the other had 55 G/M.

Bleeding. The animals were bled from the central artery of the ear, according to the technique of Leskowitz and Waksman(2). Ten ml of blood was taken at each bleeding. The first bleeding was made just before the DNP-BGG injections, the second 3 days after the third DNP-BGG injection, and then every second or third day until the 17th day after the third DNP-BGG injection.

Antibody measurement. PCA was used for antibody titrations, since it was previously shown that with rabbit antisera concordant results are obtained using PCA, passive hemagglutination and passive hemolysis(1). PCA reactions were carried out as previously described(1,3). As challenging antigen, 500 μg of BGG or DNP-BSA was used. The precautions outlined previously(1) were carefully observed. Parallel dilutions of the sera were made and the following dilutions were used: 1:10, 1:25, 1:50, 1:100, 1:200, and 1:400.

Double diffusion in agar was performed according to Ouchterlony(4).

Results and discussion. Results of the experiments are presented in the Tables.

No secondary effect against the hapten determinant was obtained in any of the experiments when the carrier protein (BGG) was injected prior to injection of the hapten-carrier protein conjugate. In fact, no anti-DNP antibody was detectable in any of the sera of the rabbits which received BGG before injection of any of the DNP-BGG preparations. Three consecutive intravenous injections of 3 mg each of the 2 preparations with the lower numbers of DNP-G/M attached (*i.e.*, 15 and 33 G/M) seemed insufficient for provoking antibody formation in any of the rabbits injected. However, it is interesting to observe that the other two, more highly coupled preparations, did provoke anti-DNP antibody production in most

TABLE II. PCA Reactions with 500 μg BGG as Challenging Antigen per Guinea Pig.

Immunization of rabbits	Titer of sera: Just before 1st DNP-BGG inj	Days after last injection of DNP-BGG: 3	5	7	9	11	14	17
BGG then DNP-BGG 15 G/M	1/25	1/200	1/200	nd	nd	nd	nd	1/25
4 rabbits (one died)	1/50	1/400	1/400	nd	nd	nd	nd	1/50
	1/100	1/400	1/400	nd	nd	nd	nd	1/100
	1/100	1/400	1/400	nd	nd	nd	nd	1/100
BGG then DNP-BGG 33 G/M	0	1/25	1/50	1/50	1/50	nd	1/25	nd
5 rabbits	0	1/25	1/50	1/50	1/50	nd	0	nd
	0	1/50	1/50	1/50	1/25	nd	1/25	nd
	1/25	1/50	1/50	1/25	1/25	nd	0	nd
	1/25	1/50	1/50	1/25	1/25	nd	0	nd
BGG then DNP-BGG 47 G/M	0	0	0	0	0	nd	0	nd
5 rabbits	0	0	0	0	0	nd	0	nd
	0	1/25	1/25	0	0	nd	0	nd
	0	1/25	1/25	0	0	nd	0	nd
	1/25	1/50	1/50	1/50	1/25	nd	0	nd
BGG then DNP-BGG 55 G/M	1/25	1/25	0	nd	nd	nd	nd	0
4 rabbits (one died)	1/25	1/100	1/50	nd	nd	nd	nd	0
	1/100	1/100	1/50	nd	nd	nd	nd	0
	1/200	1/100	1/50	nd	nd	nd	nd	0
DNP-BGG 15 G/M		0	0	nd	0	0	nd	0
5 rabbits		0	0	nd	0	0	nd	0
		0	0	nd	1/25	0	nd	0
		0	0	nd	1/25	0	nd	0
		0	0	nd	1/25	1/25	nd	0
DNP-BGG 33 G/M 5 rabbits		0	0	0	0	nd	0	0
DNP-BGG 47 G/M 5 rabbits		0	0	0	0	nd	0	0
DNP-BGG 55 G/M 4 rabbits (one died)		0	0	nd	0	0	nd	0

nd = not done.

Numbers represent the last dilution which gave positive PCA reactions. Average of at least 4 guinea pigs for each experiment.

0 = no PCA reactions even with 1/10 dilutions.

of the animals (8 out of 9). Anti-DNP antibody was first detected on the 7th day after the last injection of DNP-BGG and seemed to reach somewhat higher levels on days 9 and 11. After this interval the titer declined but antibody could be detected in one of those 4 animals which had previously demonstrated antibody production on the 17th day and which were injected with the preparation having 47 G/M. Antibody could also be detected even on the 17th day in 3 out of 4 of those animals which were injected with the preparation with 55 G/M.

Prior injection of the carrier protein not only did not result in a secondary effect, but seemed to hinder antibody formation against the DNP determinant. These results are in contrast with the observations of Salvin and Smith(5). In their experiments, there was an increase in rate of antibody formation against a heterologous hapten when the animals were previously injected with an unrelated hapten—although attached to the same carrier protein—or when the carrier protein was injected alone. However, these authors used hen egg albumin as the carrier protein, with Freund's incomplete adjuvant, and guinea pigs for antibody production. In the present study, as in that published previously(1), amount of antibody production rather than rate of antibody formation was examined and the animals used were rabbits, not guinea pigs.

Some of the animals had detectable anti-BGG antibody before injection of DNP-BGG (2 out of 5 reinjected with DNP-BGG, 33 G/M; one out of 5 reinjected with DNP-BGG, 47 G/M; and all those reinjected with DNP-BGG, 15 G/M or 55 G/M). All animals reinjected with the preparation having 15 G/M had a striking anamnestic effect on the production of anti-BGG antibodies; a less marked effect was also noted with the preparation having 33 G/M. A slight anam-

nestic effect was noticeable even with the preparations with 47 G/M (3 out of 5 had increase of anti-BGG antibodies in the 3rd and 5th day) and 55 G/M although, especially in the latter case, this effect was minimal (only one showed increased anti-BGG titers on the 3rd day after reinjection). Only the preparation with 15 G/M, when used without previous injection of BGG, provoked formation of anti-BGG antibody on the 9th day after the last injection of DNP-BGG in 3 out of 5 animals. These results are not unexpected since this preparation (15 G/M) had obviously more intact determinants characteristic for BGG than the preparations with higher numbers of DNP-G/M attached. However, in double diffusion in agar it was seen that not all natural BGG determinants even in the preparation with 55 G/M are denatured since a definite line is obtained with anti-BGG sera. It must be noted, however, that native BGG gives a much stronger line than the DNP-BGG 55 G/M preparation.

DNP-BGG does not seem to be as immunogenic in rabbits as is native BGG. In fact, only the highly coupled preparations (47 and 55 G/M) were capable of provoking antibody formation. Relatively better antibody production was observed with native BGG (higher titer of antibody) used in the same amounts as the DNP-BGG preparations.

Adler(6) recently called attention to the fact that when an animal is initially treated by injection of a single antigen and subsequently a second antigen together with the first antigen is injected, a suppression of antibody formation against the new antigen is expected. It might be that in rabbits under the conditions used for immunization (intravenous injection of the antigen with no adjuvants) competition of antigens(6) plays an important role. In this study, the native BGG determinants might be regarded as the first antigen and the DNP determinant as the second.

Summary. Rabbits were immunized with bovine gamma globulin intravenously in saline and were reinjected with dinitrophenylated bovine gamma globulin intravenously. No secondary effect on anti-dinitrophenyl antibodies was observed by prior injection of bovine gamma globulin; on the contrary, no anti-dinitrophenyl antibody was produced in these animals whereas in controls, which had not received prior bovine gamma globulin injections, antibody production was detected, especially with highly derivated dinitrophenyl-bovine gamma globulin preparations. A secondary effect against bovine gamma globulin was observed, especially with the less derivated preparation.

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