

the splenic system reacting against antigens (6).

Further purification and chemical characterization of the material responsible for the acute splenic response observed, will be of utility in clearing up the mechanism of the cellular response to purified antigens of one chemical species.

Summary. Extracts of normal rabbit urine obtained by ethanol precipitation or kaolin adsorption produce marked increase in rat spleen weight. Histologic examination of spleens shows marked hyperplasia with abundant mononuclear cells of the type described as acute splenic tumor cells. The splenic response is a function of dose of protein given. Animals treated with these extracts show significant increase in C^{14} -amino acid uptake,

specially in the α , β globulin fraction. Preirradiation of the rats (550 r Cs γ) inhibits splenic and plasma protein response to extracts of normal rabbit's urine.

1. Rich, A. R., PROC. SOC. EXP. BIOL. AND MED., 1935, v32, 1349.
2. Rich, A. R., Lewis, M. R., Wintrobe, M. M., *Bull. Johns Hopkins Hosp.*, 1939, v65, 311.
3. Wissler, R. W., Fitch, F. W., Lavia, M. F., Gunderson, C. H., *J. Cell. and Comp. Physiol.*, 1957, v50, suppl. 1, 265.
4. Winkler, R. J., in Glick: *Methods of Biochemical Analysis*. Interscience Publishers N. Y.; vII, p303.
5. Hodgson, G., Perretta, M., Yudilevich, D., Eskuche, I., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 37.
6. Wissler, R. W., Robson, M. J., Fitch, F., Nelson, W., Jacobson, L. O., *J. Immunol.*, 1953, v70, 379.

Received December 28, 1959. P.S.E.B.M., 1960, v103.

Effect of BAL (2,3-Dimercaptopropanol) on Hypoglycemic Action of Insulin. (25658)

OSCAR V. DOMINGUEZ* (Introduced by Leo T. Samuels)

Dept. de Farmacologia, E.N.C.B., Inst. Politécnico Nacional, Mexico City, D.F.

Patterson(1) described the diabetogenic action of dehydroascorbic acid (DHAA). Simultaneously, Patterson(2) and Dominguez (3) found that by injecting compounds containing sulphydryl groups, the animals were protected against the diabetogenic action of DHAA. Intravenous administration of DHAA produces a characteristic response which consists of 2 consecutive periods. The first, a violent convulsive period, starts immediately after intravenous injection of DHAA and is prolonged for no more than 3 minutes. The animals immediately enter the second period, a depressive stage of 5-10 minutes duration. The convulsive period coincides with a deep hypoglycemia. When BAL was injected intramuscularly, the rats were protected against the hypoglycemic response as well as against development of the experimental diabetes induced by dehydroascorbic acid(3). The ini-

tial hypoglycemia is probably due to a massive release of insulin from the islets of Langerhans, similar to the release of insulin which has been suggested to follow alloxan injections (4). Since BAL inhibited the initial hypoglycemia produced by DHAA, the effect of BAL on the hypoglycemia produced by exogenous insulin was studied.

Methods. Rabbits of similar weight were divided into 4 groups and fasted for 12 hours before experiment. Blood sugar was determined by Nelson's method(5) and values obtained were considered as normal control levels. Doses of insulin and BAL used in the experiment were 0.7 I.U. and 70 mg/kg of body weight, respectively. The different groups were treated as follows: *Group a* received intravenous injection of insulin. *Group b* received intramuscular injection of BAL. *Group c* were injected intramuscularly with BAL. Thirty minutes later the animals received intravenous injections of insulin. *Group d* received intravenous insulin and intramuscular BAL simultaneously. Blood glucose was

* Present address: Dept. of Biological Chemistry, University of Utah College of Medicine, Salt Lake City

TABLE I. Effect of BAL (2,3-Dimercaptopropanol) on Insulin Hypoglycemia in Rabbits.

Group	a	b	c	d
No. of rabbits	3	5	6	3
Wt in kg (avg)	2.38	2.52	2.47	2.40
Treatment	{ Insulin, IU/kg .7 BAL, mg/kg 70			
Time	Blood sugar, mg/100 ml			
0	112 (104-121)†	103 (90-115)	102 (75-120)	118 (100-121)
5 min.				90 (85-107)
10 "				98 (87-105)
20 "	98 (85-102)	106 (95-120)	106 (75-129)	65 (58-70)
40 "	75 (71-76)	109 (95-128)	123 (115-140)	85 (70-95)
1 hr	63 (60-67)	108 (90-125)	161 (130-178)	120 (117-135)
1 ", 20 min.	51 (48-57)	109 (92-130)	178 (132-225)	173 (165-178)
2 "	72 (61-101)	115 (95-135)	243 (160-376)	224 (203-249)
2 ", 20 "	70 (68-72)	111 (90-135)	255 (185-340)	280 (262-305)
2 ", 40 "	100 (92-112)	107 (90-128)	265 (192-365)	281 (260-295)
3 "	109 (103-115)	111 (92-120)	280 (238-360)	291 (265-305)

* BAL inj. (i.m.) 30 min. before insulin administration.

† BAL (i.m.) and insulin (i.v.) simultaneous administration.

‡ Figures in parentheses represent range.

determined every 20 minutes for 3 hours after insulin administration (In Group b, the zero time was the BAL injection).

Results. The results expressed in mg glucose/100 ml of blood are summarized in Table I. These values represent averages observed in the animals studied. The insulin control *Group a* showed the typical fall in blood sugar and recovery to normal values in 3 hours. In *Group b* blood glucose level was practically unaffected by BAL administration. When both BAL and insulin were administered, one would then expect either a depression in blood glucose (if BAL has no effect on insulin hypoglycemia) or continued normal glucose values (if insulin is inhibited by BAL). However, other results were obtained. In *Group c*, not only was the hypoglycemic effect of insulin not observed; a significant increase in blood sugar began 40 minutes after insulin administration (70 minutes after BAL injection), and was still slowly rising at 3 hours.

In *Group d*, the effect of insulin appeared earlier than in the control group but was not so prolonged. A definite hypoglycemia was found at 20 minutes instead of at the usual time, 1 hour to 1 hour, 40 minutes. Within one hour after administration, however, the level had returned to normal and hyperglycemia followed.

Discussion. BAL itself did not significantly affect levels of blood glucose. Perhaps levels of endogenous insulin are so low, that inversion of the response by BAL was not apparent,

or perhaps the endogenous insulin and the exogenous crystalline insulin used have different properties.

When both BAL and insulin were injected separately but at the same time (Group d), the hypoglycemic effect of insulin started, but as BAL gradually entered the circulation, inversion of the action of insulin took place. In contrast, when insulin was injected 30 minutes after BAL (Group c), it is probable that some BAL was already circulating at time of insulin injection. This group did not exhibit hypoglycemia and hyperglycemia started sooner than in Group d.

The manner in which BAL produces this effect cannot easily be explained. Huisman (6) found that BAL induced hyperglycemia in rats. Durlacher *et al.* (7), observed that in BAL poisoning, liver glycogen was decreased. Graham (8) found that the hyperglycemic effect induced by adrenaline and noradrenaline was significantly potentiated when BAL, in doses of 25 mg/kg, was previously injected intraperitoneally. The effect of adrenaline was increased 78% and that of noradrenaline 109%. Since insulin releases adrenaline (9), perhaps BAL, when present, inhibits insulin activity on the metabolism of carbohydrates but does not affect adrenaline secretion, so the hyperglycemia occurs. If this is the case, it is necessary to establish that adrenaline is secreted because of the presence of insulin, even when inactivated by BAL.

It is also possible that BAL could act by

inhibiting metabolism and inactivation of adrenaline. A further possibility is that BAL increases the release or decreases the metabolism of glucagon. The influence of BAL on the metabolism of glucagon and the catecholamines deserves investigation.

Summary. In rabbits, doses of BAL which had no significant influence on blood glucose levels caused marked hyperglycemia when injected at the same time or 30 minutes before injection of a hypoglycemic dose of insulin. Possible explanations of this effect are considered.

1. Patterson, J. W., *J. Biol. Chem.*, 1950, v183, 81.
2. Patterson, J. W., Lazarow, A., *ibid.*, 1950, v186, 141.

3. Dominguez, O. V., *Thesis for degree in Biochemistry, Escuela Superior de Ciencias Biol. (I.P.N.) Mexico*, 1950.
4. Carrasco-Formiguera, R., *Arch. de Biol. y Patol.*, 1948, vI, 111.
5. Nelson, N., *J. Biol. Chem.*, 1944, v153, 375.
6. Huisman, T. H. J., Lammers, W., Siderius, P., Hartlief, J. G., *Acta Physiol. et Pharmacol. Neerland.*, 1950, v1, 184-92.
7. Durlacher, S. H., Bunting, H., Harrison, H. H., Ordway, N. K., Albrink, W. S., *J. Pharmacol.*, 87, Suppl. 1946, 28-32.
8. Graham, J. D. P., James, D. M., *J. Physiol.*, 1952, v118, 479-85.
9. Cannon, W. B., McIver, M. H., Bliss, S. W., *Am. J. Physiol.*, 1924, v69, 46.

Received January 4, 1960. P.S.E.B.M., 1960, v103.

Interdependence of Hyaluronic Acid and M Protein in Streptococcal Aerosol Infections in Mice.*† (25659)

J. T. CUSTOD, R. I. LYTLE, B. H. JOHNSON AND P. F. FRANK
(Introduced by H. D. Slade)

*Divisions of Biochemistry and Bacteriology, Naval Medical Research Unit No. 4,
U. S. Naval Training Center, Great Lakes, Ill.*

Coburn *et al.*(1) observed that different streptococcal strains administered by aerosol vary in capacity to infect normal mice. Johnson and Furrer(2) confirmed this observation, and also reported that ability of a strain to produce certain levels of hyaluronic acid (HA) appeared related to capacity of the strain to induce aerosol infection. This report stimulated further investigation into the quantitative role of HA production in this phenomenon in presence and absence of M protein, the substance primarily associated with streptococcal virulence. An opportunity to study the latter aspect of the problem was afforded when several strains of group A streptococci were encountered which colonially re-

sembled the aerosol infective organism but which failed to possess M protein as evidenced by serological analysis.

Methods. Streptococcal strains. Twelve typable group A strains exhibiting various kinds of colonial morphology (4 glossy, 2 matt, 5 muco-matt and 1 mucoid) and the 4 non-typable group A strains which colonially resembled the other mucoid strains were all isolated from Navy recruits. *Aerosol technic.* Details regarding construction of the aerosol chamber, culture and preparations of the cell suspensions, spraying technic and reproducibility of the results produced by this technic have been described(2) and are briefly described here. Each streptococcal strain was incubated in Todd-Hewitt broth for 16 hours at 37°C. After centrifugation, the cells were washed 3 times in Gel-Locke solution and final concentration adjusted to a standard optical density value. Each group of 12 Swiss albino mice was sprayed with a streptococcal suspension twice daily for 3 days, then removed from the aerosol chamber, a throat culture taken,

* From Research Projects NM 52 06 04.4 and NM 52 06 04.7, Bureau of Medicine and Surgery, Navy Dept., Washington, D. C. Opinions and assertions expressed herein are those of the authors and cannot be construed as indicating endorsement or approval by Navy Dept. or Naval Service at large.

† A portion of this work was presented before 58th Meeting of Soc. Am. Bact., Apr. 29, 1958, Chicago.