and Connally(5), in a similar experiment evaluating the effect of steroids, showed that glucocorticoids are more effective than mineral corticoids in hemorrhagic shock, presumably augmenting the responsiveness of vessels to vasopressor drugs.

The site of action of the trauma in this experiment would appear to be largely confined to the abdomen, both by autopsy and microscopic examinations of the abdominal organs. This fact has been substantiated by Noble and Collip(4) and by Chambers, Zweibach and Lowenstein(6) who had shown that taping the abdomen or evisceration will reduce mortality. It appears that death is attributable to shock produced by trauma to the viscera. It has been postulated that a toxic substance(7,8) is absorbed and produced from the anoxic traumatized tissues and that this toxin may be the cause of shock.

The mechanism by which glucocorticoids reduce the mortality rate is not known although extensive investigations have been made. Hume and Nelson(9) have shown that operative trauma and hemorrhage produce an increase in output of 17 hydroxycorticoids and that the increase is maintained during hypotension. Further studies also suggest that in severe hypotension (below 35 mm Hg) the decreased level of corticoids may be the result of a decreased adrenal minute flow. Other experiments(10) have revealed that in severe hemorrhagic shock the adrenal blood flow remained only 15% of pre-shock levels while the output of the corticosteroids remained at 60-100% of normal levels. Fritz (11) showed that adrenalectomized rats did not exhibit the pressor response to norepinephrine and concluded that the 11 oxygenated steroids are necessary to "prime" blood vessels to constrict in response to pressor agents. In further experiments, responsiveness of one vessel was restored by topical application of adrenal cortical extracts.

Summary. We have performed an experiment using a modified Noble-Collip drum to evaluate the effect of various steroids on traumatic non-hemorrhagic shock. Our results indicate that certain glucocorticoids including hydrocortisone, prednisolone and 6α-methylprednisolone will significantly reduce the mortality of the animals subjected to the trauma.

We hereby express appreciation for technical assistance rendered by Mrs. Marija, A. Petravicius.

1. Connally, J. E., Bruns, D. L., Stofer, R. C., *Surgical Forum,* 1958, v9, 17.
2. Knapp, R. W., Howard, J. M., *Surgery,* 1957, v42, 919.
3. Howard, M., Debakey, M. E., *ibid.,* 1957, v36, 161.
4. Noble, R. I., Collip, J. B., *Physiol.,* 1942, v31, 187.
5. Bruns, D. L., Connally, J. E., *Surgical Forum,* 1959, vX, 382.
6. Chambers, R., Zweibach, B. W., Lowenstein, J. A., *American J. Physiol.,* 1943, v139, 123.
7. Fine, J., *Am. J. Gastroenterol.,* 1958, v29, 596.
8. Fine, J., Frank, H. A., Jacob, S., Gordon, T., *Ann. N. Y. Acad. Sci.,* 1952, v55, 429.
9. Hume, D. M., Nelson, D. H., *Surgical Forum,* 1954, vV, 568.
10. Howard, A., Frank, E. D., Karman, H., Machi, I. A., Hechter, O., *Am. J. Physiol.,* 1955, v182, 24.
11. Fritz, I., Levine, R., *ibid.,* 1951, v165, 456.

Received December 22, 1961. P.S.E.B.M., 1962, v109.

Effect of Cyanide and Fluoride on *in vitro* Incorporation of H^3-Cholesterol into Rabbit Artery. (27304)

JACOB SHAPIRA, JOSEPH L. GLASGOW*, WM. H. PERKINS AND CAROLYN HARVEY
(Introduced by C. F. Shukers)
Radioisotope Service, Consolidated VA Hospital, Little Rock, Ark.

The earliest lesions of rabbit intima appear within a few days after feeding large amounts of cholesterol and consist of groups of 10-50 fat-filled fibrocytes and a few histocytes lying

* Postdoctoral fellow of Nat. Inst. Mental Health, U.S.P.H.S., 1960-61.

immediately under a single layer of endothelial cells(1). Although rabbit aorta can synthesize cholesterol at a slow rate *in vitro* (2), studies with mammalian cells in culture would indicate that serum can supply most of the cellular need for cholesterol(3). Lazzarini showed(4) that serum cholesterol exchanged with tissue-bound cholesterol in human aorta *in vitro* and its synthesis has been demonstrated in human aorta by Chobanian and Hollander(5). It would be of considerable interest to determine what factors other than the cholesterol level of the serum have an effect on rate of incorporation of H^3-cholesterol into rabbit aorta. During attempts to determine the feasibility of such studies, an unexpected enhancement of the amount of cholesterol exchanged was observed in the presence of a mixture of cyanide and fluoride. This would infer that in the rabbit an energy-requiring system is necessary for exclusion of serum cholesterol from the aorta, and that its deposition in the absence of metabolic poisons or excess load would result from a decreased activity of this system.

Methods. The randomly labeled H^3-cholesterol was prepared by the exchange method of Wilzbach(6) and purified as described previously(7). The aorta of rabbits sacrificed by cervical fracture was removed rapidly, cut into 5 mm sections while wet with 20% human serum in Hanks' balanced salt solution, and incubated for 18 hours in glass test tubes (12 × 75 mm) at 36°C in an air atmosphere. The medium was composed of a suspension of 0.24 mg (12 μc) of H^3-cholesterol in 1 ml of a solution of 20% human serum in Hanks' balanced salt solution. For each experimental condition, at least 5 replicate tubes were prepared. After incubation, the tubes were drained, and the tissue was washed by centrifugation once with 5 ml of saline and 3 times with 5 ml portions of acetone. The air dried samples were weighed, placed in 20 ml counting bottles, and a solution of 3.5 g of terphenyl and 0.25 g of 1,4-di-2-(5-phenyloxazolyl)-benzene per liter of toluene was added. Overnight standing at room temperature was sufficient time to extract the maximum amount of radioactivity from the tissue into the counting solution, and the samples were counted the next day in a Packard Tri-Carb Liquid Scintillation Counter. Various other incubation conditions, times, and washing procedures were studied, but the method described gave the most consistent results.

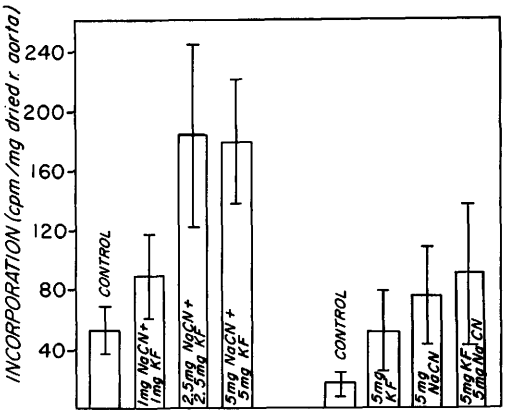

FIG. 1. *In vitro* incorporation of H^3-cholesterol into rabbit aorta.

Results. Addition of a mixture of sodium cyanide and potassium fluoride to the incubation mixture resulted in increased incorporation of radioactivity into the aorta (Fig. 1). This stimulation reached a maximum at a concentration of 2.5 mg/ml of the poison and higher concentrations had no further effect. The incorporation into the poisoned tissues was generally 3-4 times that of the corresponding controls. The rapidity with which the tissue was handled seemed to determine the absolute amount of radioactivity incorporated. Sodium cyanide was somewhat more effective than potassium fluoride in causing this stimulation of incorporation.

Several studies were performed to eliminate the possibility that the effect was due merely to differences in physical adsorption of the cholesterol from the medium. It was found that the wet weight of neither poisoned samples nor controls changed appreciably during the incubation. When samples were merely blotted dry before counting, there was no significant difference in the amount of radioactivity present in the 2 groups of samples.

It would appear that the radioactive material which accumulated in the poisoned section of aorta is appreciably soluble in nor-

mal saline since exhaustive washing with such a solution greatly reduced the amount of radioactivity in the poisoned samples. Saline washing also decreased the radioactivity in the control samples. Repeated washing with acetone did not appreciably decrease the radioactivity. When sections of aorta were held at 60°C for 30 minutes prior to incubation, their incorporation was slightly less than the control samples.

Since the radioactive cholesterol in the incubation medium was in suspension, it was decided to examine the response of the tissue to cholesterol in solution. An ether solution of 24 mg of H^3-cholesterol was added with vigorous stirring to 80 ml of Hanks' balanced salt solution containing 0.1 ml of Sterox SE (Monsanto Chemical Co., Boston, Mass.). After 4 hours of stirring and aeration, a clear ether-free solution was obtained to which was added 20 ml of pooled serum. Incorporation of radioactivity into aorta fragments from this medium was somewhat lower than when the suspension of cholesterol was used, but the same relative increase in the presence of the poison mixture was observed.

When sections of rabbit inferior vena cava were examined in the same system, no significant difference was observed between the control and poisoned samples in the amount of radioactivity taken up from the medium.

A study was performed to determine the effect of different types of serum on the incorporation. Both atherosclerotic human serum and rabbit plasma gave the same amount of incorporation as normal human serum in the absence of the poisons. The increase in incorporation due to KF or NaCN was much more pronounced in the presence of rabbit plasma than when human sera were used.

The data would indicate that prevention of the deposition of cholesterol in rabbit aorta is due to some system poisoned by cyanide and fluoride.

Summary. The *in vitro* incorporation of H^3-cholesterol into surviving rabbit aorta is increased by cyanide and fluoride. This effect is not shown by vena cava tissue. Incubation in rabbit plasma results in a greater stimulation than when either normal or atherosclerotic human serum was used. The data supports the hypothesis that an energy-requiring system functions to prevent deposition of cholesterol from the serum into the rabbit aorta.

1. Duff, G. L., MacMillian, G. C., Richie, A. C., *Am. J. Path.*, 1957, v33, 845.
2. Siperstein, M. D., Chaikoff, I. L., Chernick, S. S., *Science*, 1951, v113, 747.
3. Bailey, J. M., Gey, G. O., Gey, M. K., PROC. SOC. EXP. BIOL. AND MED., 1959, v100, 686.
4. Lazzarini, A. A., *Circulation*, 1960, v22, 656.
5. Chobanian, A. V., Hollander, W., *ibid.*, 1960, v22, 676.
6. Wilzbach, K. E., *J. Am. Chem. Soc.*, 1957, v79, 1013.
7. Doherty, J. E., Perkins, W. H., Shapira, J., *J. Lab. Clin. Med.*, 1962, in press.

Received December 22, 1961. P.S.E.B.M., 1962, v109.

Transfer and Decline of Maternal Infectious Canine Hepatitis Antibody in Puppies.* (27305)

L. E. CARMICHAEL, D. S. ROBSON AND F. D. BARNES
(Introduced by James A. Baker)
Veterinary Virus Research Institute and Biometrics Unit, Department of Plant Breeding, Cornell University, Ithaca, N. Y.

Specific immunity to infectious disease in the newborn is largely a consequence of the transfer of antibodies from the immune dam to her offspring(1,2,3). In the dog, an immune bitch bestows this immunity upon her puppies by transferring antibodies *in utero* and through colostrum(4). Passive immunity so acquired is temporary, for its dura-

* Supported by a research grant from Nat. Inst. Health, U.S.P.H.S.