agreement with dates for that event from other continents. It also establishes the Holocene age of bed VI overlying the caliche above bed V (*7*).

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References and Notes

1. L. S. B. Leakey, J. F. Evernden, G. H. Curtiss, *Nature* **191**, 478 (1961).
2. R. L. Fleischer *et al.*, *Science* **148**, 72 (1965).
3. L. S. B. Leakey, *Olduvai Gorge 1951–1961* (Cambridge Univ. Press, London, 1965), vol. 1, p. 5; and subsequent volumes.
4. K. P. Oakley, *Frameworks for Dating Fossil Man* (Aldine, Chicago, 1966), pp. 176 and 192.
5. R. Berger, A. G. Horney, W. F. Libby, *Science* **144**, 999 (1964).
6. G. H. Curtiss, personal communication.
7. R. Berger and W. F. Libby, *Radiocarbon*, in press.
8. We thank W. F. Libby for encouragement. Supported by NSF grant GA-628. Publication 682 of the Institute of Geophysics, U.C.L.A.

5 July 1968

Ouabain Hypoglycemia: Insulin Mediation

Abstract. *The fall in blood sugar occurring during infusion of ouabain (1 microgram per kilogram per minute for a 60-minute period) in dogs is accompanied by an increased uptake of glucose and potassium by the liver. Concurrently, plasma insulin in the portal blood increases significantly. This increase appears to be a result of increased insulin secretion caused by ouabain.*

The hypoglycemic effect of ouabain in the dog has been described (*1*). This hypoglycemia was reportedly more marked when ouabain was combined with insulin than when either drug was administered separately (*2*), and it was not present in pancreatectomized animals (*3*). The purpose of our experiments was to determine whether there is an interaction of ouabain with the insulin in the blood, or whether ouabain causes an increase in plasma insulin levels.

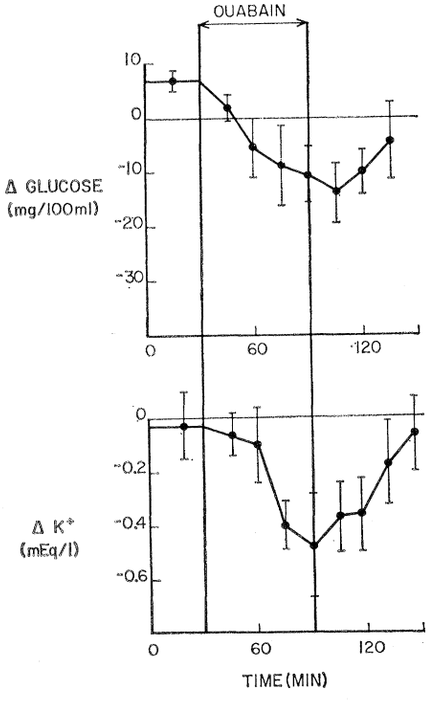

Fig. 1. Differences in glucose and potassium concentration between plasma of portal and hepatic vein blood in six dogs after intravenous administration of ouabain (1 μg kg^{-1} min^{-1} for 60 minutes).

Laparotomies were performed on 12 mongrel dogs, anesthetized and prepared as described (*4*). A polyvinyl catheter was then placed into the portal vein through a branch of gastrosplenic vein without disturbing the stem of the portal vein. Another catheter was inserted under fluoroscopy through the right jugular vein into a hepatic vein. Catheters were also placed in a femoral vein and artery. After a control period of 30 minutes, ouabain (1μg kg^{-1} min^{-1}) was administered intravenously (femoral vein) for 60 minutes. The experiment was continued for 60 minutes after the end of ouabain administration. Blood samples were taken at regular intervals from portal vein, hepatic vein, and femoral artery for measurements of the concentrations of glucose, potassium, and insulin. The insulin was quantitated by radioimmunoassay (*5*) and the result is expressed in terms of a porcine standard (*6*) by the use of a guinea pig antiserum which reacted essentially identically with porcine and canine insulin.

After ouabain administration, the glucose in arterial blood decreased from 103 to 80 mg per 100 ml (−23 percent, $P < .05$). The portohepatic difference (+6 mg per 100 ml) in plasma glucose during the control period indicates a net output, as expected in fasting animals. After ouabain administration, this trend was reversed. The portohepatic difference, amounting to −13.6 mg per 100 ml ($P < .05$), indicates a net uptake of glucose by the liver (Fig. 1). Changes in the concentration of potassium in the plasma paralleled the changes in glucose concentration: the portohepatic difference increased significantly in comparison with control (− 0.4 meq/liter, $P < .05$). Other results indicating the role of insulin in the mediation of the hypoglycemia caused by ouabain have been reported (*3*).

The amount of insulin in the plasma of the portal blood during ouabain infusion increased significantly (by 125 percent, $P < .05$) in comparison with the concentration in plasma of portal blood of the control group (Fig. 2). An hour after the end of the ouabain infusion, plasma insulin of portal blood tended to return to control values.

In the plasma of arterial blood of controls, the initial concentrations of insulin were lower than those found in the plasma of portal blood, as expected from the inactivation of insulin by the liver (*7*). The increase of plasma insulin in peripheral blood after ouabain administration, although consistent, was lower (increase of 77 percent as compared to 125 percent) than that observed in portal blood. These observations indicate that the increased amount of insulin in the plasma during ouabain administration is not due to a change in

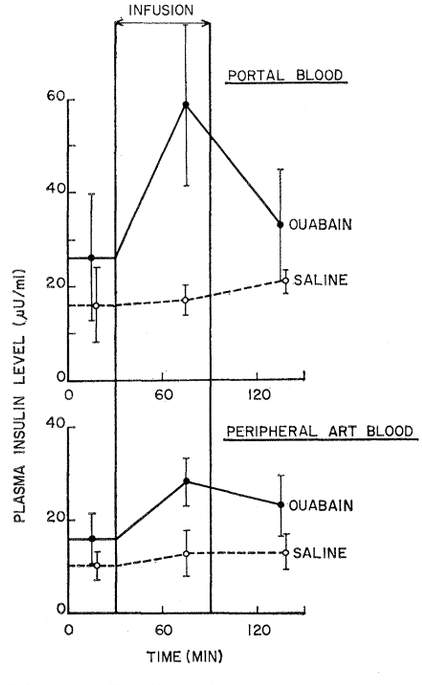

Fig. 2. Insulin plasma concentrations (mean ± S.E.) in arterial and portal blood of six dogs after intravenous administration of ouabain (1 μg kg^{-1} min^{-1} for 60 minutes) and in six control dogs receiving saline at the same rate. Concentration of insulin plasma increased significantly ($P < .05$) in portal blood at the end of ouabain infusion.

the rate of inactivation of insulin by the liver but appears to be a result of an increased secretion of insulin. This conclusion is in agreement with Milner's report (*8*) that $10^{-5}M$ ouabain stimulated insulin secretion in pancreatic tissue in vitro. The exact mechanism of this action of ouabain must still be clarified. Whether it is related to an effect of ouabain on sodium and potassium active transport, as postulated by Milner, or to an effect of the sugar molecule which is part of the glycoside, remains to be established.

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References and Notes

1. L. Triner, J. Kypson, G. G. Nahas, *Proc. Soc. Exp. Biol. Med.* **127**, 1255 (1968).
2. ———, *Proc. Int. Congr. Physiol. 24th Washington*, **7**, 440 (1968).
3. G. G. Nahas, L. Triner, J. Kypson, J. Papayoanou, *Pharmacologist* **10**, 200 (1968).
4. G. G. Nahas and C. Poyart, *Amer. J. Physiol.* **212**, 765 (1967).
5. R. S. Yalow and S. A. Berson, *J. Clin. Invest.* **39**, 1157 (1960).
6. Supplied by Eli Lilly and Company (lot PJ-5589).
7. G. E. Mortimore and F. Tietze, *Metabolism* **8**, 479 (1959).
8. R. D. G. Milner and C. N. Hales, *Biochim. Biophys. Acta* **135**, 375 (1967).
9. We thank I. Bao and P. Berryer for technical assistance. Supported, in part, by U.S. Army contract DADA-17-67-C-7126, National Institutes of Health grant GM-09069-05, and Public Health Service grant AM-11-294. P.K. holds Public Health Service grant TI AM-5397. L.T. is an established investigator of the American Heart Association.

8 August 1968

Autoimmune Chorioretinitis in Rhesus Monkeys

Abstract. *Monkeys injected with monkey retinal tissue incorporated in Freund's complete adjuvant developed ocular lesions characterized by choroiditic patches in the fundus periphery and sheathing of retinal vessels. Bovine retina, monkey choroid plexus, and guinea pig kidney were ineffective in this respect.*

An unusual complication of experimental autoimmune encephalomyelitis (AE) has been described in rhesus monkeys; the lesion, a hemorrhagic retinopathy (HR) occurs during the early stages of the AE (see *1–3*). A matter of primary consideration was whether the retinal damage represented an intrinsic feature of the AE syndrome, or an indirect and secondary complication of AE, or a separate disease entity. The possibility that central nervous system and retina could share common antigens provided the first point for experimental attack; another possibility was that the vascular damage in the retina was a response to sensitization with antigens of vascular origin rather than to central nervous system or retinal antigen.

Ten juvenile rhesus monkeys (*Macaca mulatta*) of both sexes were given a single immunizing dose of 100 mg (wet weight) of freshly dissected and emulsified monkey retina incorporated in Freund's complete adjuvant containing 0.5 mg of dried, killed *Mycobacterium tuberculosis*. The total volume of 0.5 ml of emulsion was injected into four sites subcutaneously over the scapular and nuchal regions. The animals were housed and fed as described (*1–3*) and examined at frequent intervals for signs of central nervous system disease and for eye lesions. Control groups of rhesus monkeys of equivalent age received the following tissues emulsified in Freund's complete adjuvant and injected in similar fashion: monkey choroid plexus (16 to 40 mg) or guinea pig kidney or spinal cord (0.25 ml of 50 percent suspensions). Other control monkeys received the complete adjuvant without antigens. In experiments to determine the species specificity of retinal antigen, bovine retinal tissue (100 mg) was substituted for that of monkey (*4*). After retinal lesions developed, histological examination was performed on the eyes and optic nerves.

Monkeys immunized with monkey retinal tissue developed ocular lesions in all instances, whereas bovine retina, monkey choroid plexus, and guinea pig kidney were ineffective. The first clinical signs of ocular disease consisted of choroiditic patches of varying size in the fundus periphery and sheathing of retinal vessels. There was no clear correlation between the extent of the lesions in the two tissues. In three eyes, a hemorrhagic component complicated the picture, and occasionally lid or periorbital edema and conjunctival hyperemia preceded the onset of fundus changes. Histologic examination established the presence of perivasculitis in the retina and mild or severe uveitis, especially located in the pre-equatorial portion of the choroid (Fig. 1). The optic nerve was free of inflammatory changes with the exception of the large vessels on the disk which in several instances showed a moderate accumulation of lymphoid cells in the adventitial tissue (*5*).

The lesions produced in the monkey eye by immunization with homologous retinal tissue differed sharply from those produced with guinea pig spinal cord. Immunization with this heterologous antigen resulted often in destructive hemorrhagic vasculo-occlusive retinopathy without perivasculitis, minor involvement of the uvea, and the typical widespread inflammation in the optic nerve.

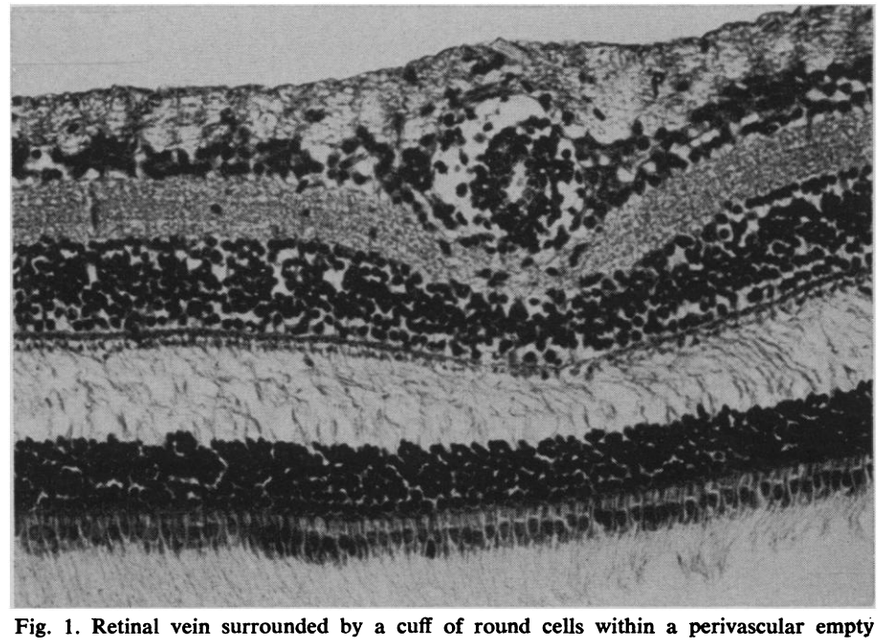

Fig. 1. Retinal vein surrounded by a cuff of round cells within a perivascular empty space.