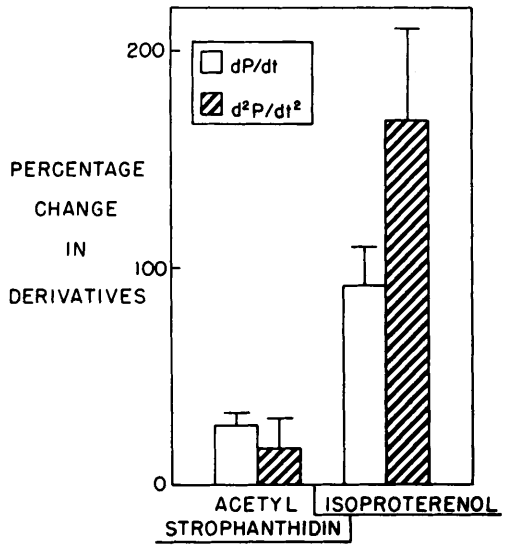

FIG. 4. Percentage change in dP/dt and d^2P/dt^2 with infusion of acetyl strophanthidin and isoproterenol. For acetyl strophanthidin, measurements made at ninth minute of infusion of 100 micrograms/min. For isoproterenol, measurements made at second minute of infusion of 9 micrograms/min. Data presented as mean ± SEM. Both derivatives increased significantly over the control value with isoproterenol infusion; the increase in d^2P/dt^2 was significantly greater than dP/dt ($p < .05$). With acetyl strophanthidin infusion, only dP/dt showed a significant increase over the control value ($p < .05$).

action of digitalis on the myocardial contractile apparatus differs in some fundamental way from that of other inotropic stimuli.

Summary. A new high fidelity fiberoptic pressure catheter has been used to measure the second derivative of left ventricular pressure, which constitutes an index of myocardial contractility. It may provide new information about the early phase of isometric ventricular contraction.

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Received June 12, 1967. P.S.E.B.M., 1968, Vol. 127.

Changes in Carbohydrate Metabolism of Squirrel Monkeys with Chromium Dietary Supplementation* (32622)

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A high prevalence of an impairment of carbohydrate metabolism is found in squirrel monkeys maintained on a standard commercial chow under controlled laboratory conditions (1,2). The animals are asymptomatic of diabetes but exhibit abnormal diagnostic tolbutamide and glucose tolerance tests. Mertz and his co-workers have shown that progressive impairment of glucose tolerance associated with a diminished response of isolated tissues to insulin *in vitro* develops in rats and humans maintained on suboptimal intake of trivalent chromium (3). The metabolic defect was reversed by supplementation of trivalent chromium in drinking water or diet. Analysis (8) of the diet and drinking water of the squirrel monkeys revealed that the total chromium content was relatively low and of unknown valency state. The present investigation was undertaken to determine if the abnormal diagnostic tolbutamide responses characteristic of metabolically impaired squirrel monkeys could be reversed and their glucose tolerance improved with chromium (III) dietary supplementation.

Methods and Materials. The squirrel monkeys from the colony used in these experiments

* This study was supported in part by NIH grants FR-00180-01 and CA-06474.

were females of uncertain adult age (600–800 gm) obtained from Tarpon Zoo, Tarpon Springs, Florida, within 2–4 weeks of capture at Leticia, Colombia. The animals were examined for disease and stabilized for at least 4 weeks under colony conditions. They were maintained on Purina Monkey Chow 25 (Ralston Purina Co., Saint Louis, Mo.). The approximate composition of the chow is crude protein 26.8%, carbohydrate 49.5%, fat 4.9%, fiber 2.7%, vitamin supplement and added minerals (4.31 kcal/gm). Complete elemental analysis supplied by manufacturer was Ca 0.96, P 0.56, K 1.03, Mg 0.12, and Na 0.35%; Fe 244, Zn 21.1, Mn 40.5, Cu 13.4, Co 0.25, and I 0.81 ppm. Chromium analysis[1] by atomic absorption spectroscopy (8) of two batches of chow gave a mean content of 3.3 ppm dry weight. The animals were housed 2 to a standard stainless steel cage at an environmental temperature of 27°C.

Monkeys were selected for the experiments on the basis of their responses to the oral glucose tolerance test as modified by Lang (2) and the diagnostic tolbutamide test according to criteria described previously(1). During the experiments the animals were fed their standard maintenance diet *ad libitum* and provided drinking water from Pyrex glass reservoirs preequilibrated with chromium solutions. The dietary intake of chromium was supplemented by the addition of 10 ppm reagent grade chromium(III) acetate or chromium(II) chloride to ordinary drinking water which contained less than 0.03 ppm.[1] The drinking solutions were prepared fresh daily from stock solutions of chromium kept at 4°C. Chromium analysis (8) of aliquots from drinking solutions removed from water bottles after 24 hours showed negligible loss (4%) by glass adsorption or precipitation from chromium(III) solutions at pH 7.1 or 4.8, although a 40% loss from chromium(II) solution was noted. The daily water consumption was 100–125 ml per animal and was unaffected by chromium addition.

The standard intravenous tolbutamide diagnostic test was carried out under sodium amobarbital anesthesia (50 mg/kg, ip) after a fast of 15–18 hours. The anesthetic produced complete muscle relaxation within 8–10 min after which the blood glucose level remained stable for at least 4 hours. Sodium tolbutamide[2] (15 mg/kg) was given via the external jugular vein 45 min after the induction of anesthesia. Blood samples for glucose determinations were drawn from the contralateral jugular vein. A high-dose intravenous glucose tolerance test (4) was performed under similar conditions. Sterile glucose (75 mg/kg) was given via the external jugular vein and blood samples were obtained from the contralateral jugular vein at 10, 20, 40, and 60 min. Blood glucose was determined by the Somogyi–Nelson method (5). The blood glucose removal rate k was calculated according to Lundbaek (6). Serum insulin levels of fasting conscious monkeys were determined using the double antibody radioimmunoassay of Hales and Randle (7) and standardized with crystalline bovine and rhesus monkey insulins.[3]

Results and Discussion. The metabolism of squirrel monkeys from the colony was evaluated with diagnostic tolbutamide and glucose tolerance tests, and 9 animals, 5 impaired responders and 4 normal responders (Fig. 1A), were chosen for investigating the effect of supplemental trivalent chromium on these metabolic states. Before supplementation, the daily chromium intake from food (commercial monkey chow) and water of all valency states averaged 138 μg per animal as determined by the analytical method used (8). Supplementation with chromium (III) to the drinking water (10 ppm) increased the intake to approximately 1568 μg per animal per day. During the experimental period, the daily food intake of impaired and normal animals was not significantly different (mean, 44.2 ± 1.6 gm per monkey per day), and the individual body weights showed little change (initial 687 ± 36 gm; final 693 ± 31 gm).

After an initial 3-week period of increased chromium(III) intake given in drinking water

[1] Chromium analysis of diet and drinking water was carried out through the generosity of Dr. James Dobbins, Research Department, R. J. Reynolds Tobacco Company, Winston-Salem, N. C.

[2] Sodium tolbutamide (Orinase) for intravenous use was generously supplied by Dr. Russel Poucher, The Upjohn Company, Kalamazoo, Mich.

[3] Crystalline rhesus monkey insulin was the kind gift of Dr. A. M. Fisher, Connaught Medical Research Laboratory, University of Toronto.

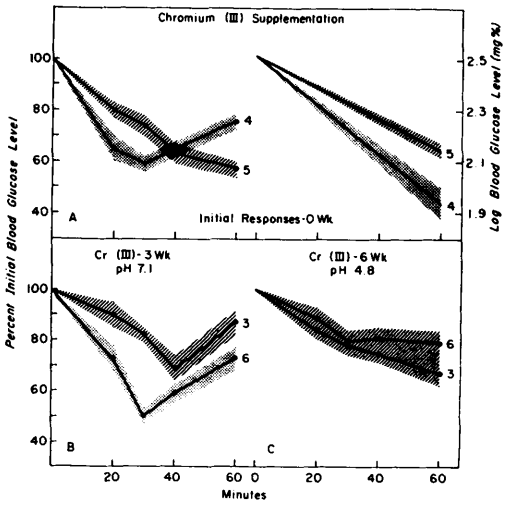

FIG. 1. The effect of dietary supplmentation with chromium(III) acetate (10 ppm in drinking water) on the responses of squirrel monkeys to diagnostic tolbutamide and intravenous glucose tolerance tests: A, initial responses to diagnostic tolbutamide and regression of blood glucose with time after intravenous glucose load of 9 animals (4 normal and 5 abnormal); B, tolbutamide responses after 3 weeks chromium(III) in water pH 7.1 (6 normal and 3 abnormal); C, tolbutamide responses after 6 weeks chromium(III) in water pH 4.8 (9 abnormal). The shaded areas represents the range of ± 1 standard error of the mean tolbutamide response and 95% confidence limits for the coefficients of the regression for glucose tolerance.

at neutral pH, the response to the tolbutamide test of the normal animals was found to be exaggerated. While the response pattern of the impaired monkeys was improved also, only one animal was restored completely to normal (Fig. 1B). This mildly beneficial effect was consistent with the improvement in glucose tolerance noted by Mertz *et al.* in rats and humans (9); and their observations that trivalent chromium enhances the action of insulin on muscle and adipose tissue (3) since a normal tolbutamide response is also dependent on the assimilation of endogenous glucose as well as pancreatic insulin secretory capacity. Because Glinsmann and Mertz (10) reported that acid-stabilized chromium solutions were more efficacious for improving glucose assimilation, for the next 6 weeks the animals were given the chromium(III) in drinking water at pH 4.8. Retest of the monkeys after this period revealed a complete conversion of previously normal monkeys to metabolically impaired animals as evaluated by the tolbutamide test (Fig. 1C) and by glucose tolerance tests. This result was inconsistent with the beneficial effects of chromium(III) on glucose tolerance noted by Glinsmann and Mertz. Although they found that a mild transient increase in impairment may often precede marked improvement of tolerance, conversion of normal glucose tolerance to abnormal was not observed by these investigators (10).

After a further 2 weeks of acid-chromium (III) supplementation, the monkeys were again tested, and since the tolbutamide and glucose tolerance tests remained abnormal (Fig. 2A), they were therefore replaced on chromium(III)-supplemented (10 ppm) drinking water at neutral pH. A control group of 6 monkeys from the colony with normal responses to these tests (Fig. 2B) were given divalent chromium(II) (10 ppm) in drinking water at neutral pH. During the following 22-week period, the food intake of the two groups of monkeys was equivalent (45.1 ± 2.1 and 43.8 ± 1.8 gm per day per monkey, respectively) and their body weights did not change significantly. The chromium(III) sup-

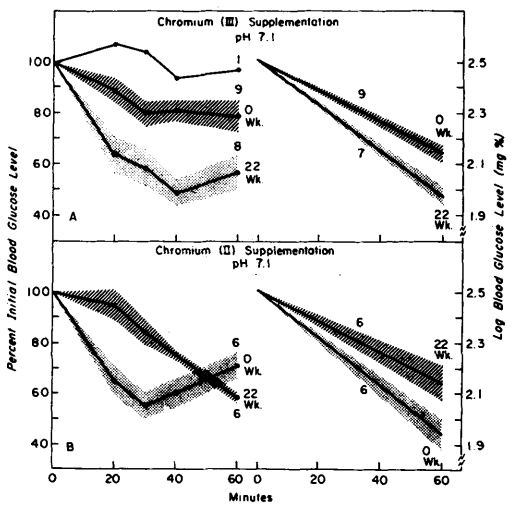

FIG. 2. The effect of dietary supplementation for 22 weeks with A. chromium(III) acetate and with B. chromium(II) chloride (10 ppm in drinking water) on the responses of squirrel monkeys to diagnostic tolbutamide and intravenous glucose tolerance tests. The shaded areas represents the range of ± 1 standard error of the mean tolbutamide response and 95% confidence limits for the coefficients of the regression for blood glucose level with time after intravenous glucose load.

plemented monkeys were tested for response to tolbutamide at 5- to 6-week intervals. Their response to this test improved gradually until, at 22 weeks, 8 of 9 animals had reverted to normal (Fig. 2A). Moreover the mean assimilation rate of an intravenous glucose load k showed a marked increase from 1.38 $\pm$ 0.03 to 2.33 $\pm$ 0.03 (Fig. 2A). The single animal that did not respond to trivalent chromium remained abnormal also to the intravenous glucose tolerance test. The initial mean fasting serum insulin level of the monkeys (18.2 $\pm$ 1.4 μU/ml) did not change significantly with improvement in glucose metabolism (19.8 $\pm$ 2.7 μU/ml). In contrast to these findings, 22 weeks of chromium(II) supplementation to normal monkeys produced metabolic impairment in these animals (Fig. 2B). In addition to markedly abnormal responses to tolbutamide, comparison of the glucose assimilation rate k for these animals before (2.32 $\pm$ 0.20) and after supplementation (1.56 $\pm$ 0.11) and with those given chromium(III) (2.33 $\pm$ 0.30) revealed a significant decrease in glucose tolerance ($p<.05$) (Fig. 2B). Nevertheless the fasting serum insulin levels (19.2 $\pm$ 1.2 μU/ml) were not different from normal untreated monkeys (22.1 $\pm$ 2.5 μU/ml).

These results show that colony monkeys classified as abnormal in glucose metabolism were eventually restored to normal by supplemental trivalent chromium at neutral pH despite intervening impairment with acid-stable chromium(III). The improvement in carbohydrate metabolism produced by supplemental trivalent chromium in drinking water appeared to be dependent on the pH of the solution. Monkeys with normal responses to the metabolic tests were unchanged by the administration of chromium(III) at neutral pH, but when given chromium(III) at acid pH became impaired. This impairment was overcome again by changing the pH of the chromium solution to neutral. Periodic testing over extensive intervals has shown that these alternating metabolic states induced by chromium supplementation at neutral or acid pH did not occur for animals of the colony maintained on ordinary drinking water (1). The apparent importance of the mode of administration of trivalent chromium and the unexpected deleterious effects of divalent chromium are not immediately clear. Mertz and co-workers found that trivalent chromium given by the oral route was absorbed from the gastrointestinal tract in the amount of only a few percent. This small fraction after transfer across the intestinal wall appeared in the blood firmly bound to siderophilin, the carrier protein for the internal chromium pool (10, 11). However, Donaldson and Barreras (12) found that the small percentage absorption from the gastrointestinal tract of orally administered chromium was related to an extensive binding to gastric contents in acid medium and that greater absorption was obtained when chromium solutions in alkaline medium were administered directly into the duodenum. The efficacy of the oral trivalent chromium chemical complex to correct a deficiency state is probably related to the amount of chromium absorbed into the internal pool. Therefore it seeems possible that for squirrel monkeys greater intestinal absorption occurred when chromium(III) acetate was given in drinking water at neutral pH and when the chromium solution was taken between eating period while the gastric acid content was minimal. As observed by other investigators (10), the improvement in carbohydrate metabolism was not evident immediately with increased chromium(III) intake. Although mild improvement was noted in some animals within 3 weeks, a period of 22 weeks of supplementation was required to reverse the impairment. This delay in response may be related to the amount of absorption and the rate of turnover of the internal chromium pool. The observation that superordinary amounts of chromium(II) chloride given orally produced marked impairment in carbohydrate metabolism suggests that divalent chromium may interfere with the absorption or the effect on glucose metabolism of trivalent chromium, the biologically active form of chromium at the cellular level (3).

Summary. Adult squirrel monkeys maintained on a commercial chow demonstrated an impaired carbohydrate metabolism as evaluated by diagnostic tolbutamide and glucose tolerance tests. The possibility that the diet was deficient in trivalent chromium was investigated. The diet of normal and impaired

monkeys was supplemented by the addition of trivalent and divalent chromium to the drinking water. The trivalent chromium supplementation produced an improvement in the tolbutamide and glucose tolerance responses of impaired monkeys when the drinking water was maintained at neutral pH but not at mildly acid pH. Normal monkeys supplemented with divalent chromium resulted in the appearance of impaired responses to tolbutamide and glucose tolerance tests.

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Received June 19, 1967. P.S.E.B.M., 1968, Vol. 127.

Relation of Mammotropes to Mammary Tumors VI. Immunoassays of Rat Prolactin and Growth Hormones* (32623)

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Experiments described in earlier communications (1–4) indicate that hormones of the pituitary mammotropes (Mt) play a determining role in the induction and progressive growth of mammary tumors of the rat. These cells are commonly called "acidophiles." It is generally believed that acidophiles can secrete both growth hormone (GH) and prolactin (P). A review of experimental data (5) led us to conclude that a single cell can secrete both types of hormones. Until recently these hormones were identified by bioassays only. More recently acrylamide gel electrophoresis led to recognition of the P and GH profiles (6). Immunoprecipitation (7) and radioimmunoassays (8) enable simple quantitation of rat GH and P.

Presently we shall describe the development of anti-rat prolactin (a. rP) sera, the immunologic distinctness of rat P and GH and the use of these antisera to quantitate P and GH in pituitary extracts and in organ cultures of pituitaries.

Materials and Methods. The purified rat GH was obtained from Dr. A. E. Wilhelmi (R 86567, containing 2 U/mg; designated rGH/W) and from Dr. S. Ellis (GH-II-23-B; designated rGH/E).

Purified rat P was obtained from Dr. R. Bates (RB 18-115C; 1.6 U/mg; designated P/B and from Dr. S. Ellis (XLVI-35C; designated P/E.).

Antisera were prepared by immunization of rabbits as described previously (7). The quantity of antigen used was kept close to the threshold in order to minimize antibody production by minor antigenic components present in the material injected into rabbits. For similar reasons the rabbits were exsanguinated as soon as a satisfactory precipitation was obtained with the antigen under study.

Organ cultures were set up according to the technique of Nicoll and Meites (9). Fragments of normal female rat pituitaries, enlarged pituitaries of rats given diethyl-

* Supported by grant 06215 of the Nat. Cancer Inst.

[1] Fellow of the Damon Runyon Memorial Fund.