turally to cholesterol, has been characterized in terms of its effects on acetate and mevalonate metabolism *in vitro*. The compound did not reduce acetate conversion to CO_2, fatty acids, or keto-acids but did inhibit incorporation of this substrate into cholesterol. Inhibition of mevalonate conversion to cholesterol was less than when acetate was the substrate. The similarity of the effects of SC-11952 to those produced by cholesterol feeding is discussed.

1. Siperstein, M. D., Guest, M. J., *J. Clin. Invest.*, 1959, v38, 1043.
2. ———, *ibid.*, 1960, v39, 642.
3. Bucher, N. L. R., *J. Am. Chem. Soc.*, 1953, v75, 498.
4. Hirsch, J., Ahrens, E. H., *J. Biol. Chem.*, 1958, v233, 311.
5. Kronfeld, D. S., Kleiber, M., *J. Appl. Physiol.*, 1959, v14, 1033.
6. Bucher, N. L. R., McGarrahan, K., Gould, E., Loud, A. V., *J. Biol. Chem.*, 1959, v234, 262.
7. Woolley, D. W., *Science*, 1959, v129, 615.

Received December 15, 1961. P.S.E.B.M., 1962, v109.

Water and Electrolyte Pharmacodynamics of Aspirin.* (27347)

R. W. GARDIER,† R. STEEN,‡ AND A. B. RICHARDS (Introduced by F. W. Hughes)
Departments of Pharmacology and Anesthesiology, Indiana University School of Medicine, Indianapolis

Acetylsalicylic acid (aspirin) was introduced into therapy by Dreser in 1899(1) and has since occupied a prominent position as an analgesic and antipyretic. Although aspirin analgesia is difficult to evaluate and explain, the antipyretic action has been resolved, tentatively, to increased heat loss *via* hydremia, vasodilation, and sweating(2,3,4). On this basis an assessment of aspirin-induced alterations of relatively simple parameters of fluid biodynamics would not only contribute to the pharmacology of the drug but also might provide a test for aspirin-like substitutes. Consequently, this study concerns the effects of aspirin on urinary sodium, potassium and volume and extra-cellular fluid space in influenza-infected and non-infected animals.

Methods. Male albino Webster Swiss mice (20-30 g) were hydrated with 5 ml of distilled water per 100 g of body weight *per os*. Aspirin dissolved in the hydration fluid was given in a dose of 10 mg/kg of body weight (0.02% solution for proper volume). Dilute acetic acid (placebo) having a pH (3.2) comparable to the aspirin solution was given to some animals instead of distilled water. Prior observations indicated that starvation for 16-24 hours before testing was unnecessary.

Immediately following hydration, urine was collected in 15 ml centrifuge tubes for 3 hours as follows: Silicone lubricant was applied to the inner surface of 10 glass funnels (diam. 150 mm, stem length 150 mm) held in a suitable rack. Watch glasses (diam. 100 mm) were treated similarly with silicone and each inverted and fixed to the inside of a funnel at 3 points with paraffin on surgical adhesive tape so that it rested at a slight angle. The fit of the watch glass and funnel sides allowed passage of urine but not feces. Three mice were placed in each funnel which was then covered with a watch glass (diam. 150 mm) and secured with tape. Urine volume was measured and specimens refrigerated in stoppered plastic tubes for Na^+ and K^+ determinations (Coleman Model 21 flame photometer).

Thiocyanate spaces also were calculated as a relative estimation of extracellular fluid volume(5,6). Each mouse received 0.5 or 0.75 mg of Na thiocyanate i.v.. Serum thiocyanate concentrations were determined at the end of the first, second and third hours on

* This work was partially supported by grant from P.H.S.

† Present address: Dept. of Pharmacology, Univ. Texas Medical Branch, Galveston.

‡ Supported by National Fund for Medical Education Grant.

TABLE I. Fluid and Electrolyte Studies in Mice.

	Non-infected control	Non-infected aspirin, 10 mg/kg	Infected control	Infected aspirin, 10 mg/kg
Urine vol (ml/100 g)	5.38 ± .08*(60)†	5.05 ± .20 (60)	4.34 ± .15 (58)	3.99 ± .34 (60)
Urine Na^+ (μeq/100 g)	223 ± 23.3 (60)	201 ± 18.0 (60)	90 ± 16.0 (58)	53 ± 14.8 (60)
Urine K^+ (μeq/100 g)	128 ± 16.0 (60)	99 ± 12.0 (60)	145 ± 13.0 (58)	115 ± 17.0 (60)
Thiocyanate space (ml/100 g)	35.8 ± 7.97 (45)	35.4 ± 9.19 (30)	30.6 ± 3.37 (45)	18.9 ± 2.35 (45)

* Mean ± stand. error. † No. of mice.

samples pooled from 5 control as well as from 5 test mice. These were obtained by severing the axillary vessels and collecting blood from the pouch formed by the skin incision.

Animals were inoculated with mouse lung adapted influenza virus (Type A PR-8) in a 5-day LD_{50} dose. An increased post mortem lung-body weight ratio was used to confirm the presence of infection.

Experiments were conducted simultaneously on a control group (distilled water or placebo—15 mice) and a test group (aspirin —15 mice). Since all analyses were performed on pooled samples, statistical evaluations were based on 15 mice = n of 1. All changes, from respective controls, not indicated as significant, had P values >0.05. Infected mice were tested during the first 4 days following inoculation. No correlation was found between age of infection and urinary measurements.

Results and discussion. Each value in Table I represents a mean of 30-60 mice. Urinary volumes were significantly(7) reduced, $P<0.05$, in the presence of infection. Aspirin treatment *per se* effected decreases in urine output from the respective control value. All changes in urine volume apparently were a consequence of electrolyte excretion. Na^+ excretion was significantly, $P<0.01$, reduced by infection and further decreased with aspirin treatment. Urinary K^+ also was lowered by aspirin so that the overall renal response following this drug was one of electrolyte and water conservation. Infection increased the loss of K^+, simultaneous with Na^+ retention and presumably indicates adrenal cortical activation. Aspirin opposed the K^+ loss and enhanced the effect on Na^+. This aspirin-induced conservation of K^+ as well as Na^+ substantiates a report(8) that the drug action is not mediated through the adrenal cortex.

Fever and aspirin antipyresis supposedly effect a hydremia(2,3,4). The significant reduction ($P<0.05$) in thiocyanate space in infected animals receiving aspirin implies that the urinary responses to aspirin were not due to a direct renal effect, but are suggestive of a dehydration as might occur from sweating. However, sweating does not occur in mice. Respiratory water loss was not responsible since the dose of aspirin used avoided toxic hyperpnea and is comparable to that used in human therapy. It is reasonable to assume that the thiocyanate spaces in the normal animals were higher than the true extracellular fluid volume because of thiocyanate diffusing into cells, and that aspirin had decreased this cellular permeability. Such an explanation has precedence from earlier work on antihyaluronidase activity of salicylates(9). Yet this rationale is untenable because: (a) the thiocyanate space was not increased by infection *per se* when such a phenomenon might be expected(10), (b) changes in electrolyte and water excretion in infected animals, especially with aspirin, were not consistent with decreases in cell permeability.

The problem of infection with related dynamics of intra- and extracellular water, Na^+ and K^+ requires further investigation. Certainly the aspirin effect is apparent. This drug reduced K^+ loss and produced a renal conservation of Na^+ and water, presumably by mechanisms directed toward cellular hydration at the expense of the extracellular fluid.

Elevating the dose of aspirin to 20 and 40 mg/kg resulted only in increasing the variability of the urinary data. Electrolyte excretory patterns were little altered from control or were such to imply renal changes compensatory to possible respiratory stimulation.

Summary and conclusions. Aspirin (10 mg/kg) administered *per os* to influenza-infected and non-infected mice reduced urinary excretion of Na^+, K^+ and water. This evidence indicates that aspirin is not adrenal corticoid in nature. In the infected animal aspirin also decreased the thiocyanate space which thus precludes a direct renal action for the urinary changes observed. Evidently aspirin activates mechanisms favoring expansion of intracellular fluid volume at the expense of extracellular fluid.

Acknowledgment is made to Dr. A. S. Levine, Department of Microbiology, for supplying the virus used in this study, and to I. Womak, Department of Physiology, for the Na^+ and K^+ determinations.

1. Dreser, H., *Arch. ges. Physiol.*, 1899, v76, 306.
2. Barbour, H. G., *Arch. int. Med.*, 1919, v24, 624.
3. Barbour, H. G., Herrmann, J. B., *J. Pharmacol.*, 1921, v18, 165.
4. Barbour, H. G., Guerra, F., *ibid.*, 1943, v79, 55.
5. Crandall, L. W., Anderson, M. X., *Am. J. dig. Dis.*, 1934, v1, 126.
6. Eder, H. A., *Methods in Medical Research,* The Year Book Publishers, Inc., Chicago, Ill., 1951, v4, 48.
7. Snedecor, C. W., *Statistical Methods,* 6th Ed., Iowa State College Press, Ames, 1956, 237.
8. Ingle, D. J., *J. Amer. Pharm. Assn.* (Sci. Ed.), 1953, v42, 247.
9. Guerra, F., *Science,* 1946, v103, 686.
10. Overman, R. R., Davis, A. K., Tharp, E., *Fed. Proc.*, 1947, v6, 174.

Received December 18, 1961. P.S.E.B.M., 1962, v109.

Lipase Activity in the Chick Liver during Development. (27348)

J. C. GEORGE AND P. THOMAS IYPE (Introduced by D. S. Farner)
Division of Animal Physiology, Department of Zoology, M. S. University, Baroda, India

Fat is considered to be the predominant energy source in avian development. About 60% of the total fatty acids present initially in the egg is oxidized from which the embryo derives over 90% of its caloric requirements (1). Though much work has been done on enzymes during development, either in the whole chick embryo or in the different organs of the embryo, relatively little quantitative information is available on the day-to-day fluctuations in concentrations of the enzyme, lipase, during development. Gavialo (2,4) showed that lipase is present on the first day of development of the chick embryo. Gavialo and Goryukina(3,4) later observed increasing lipase activity in the liver about the 15th day of incubation. Recently George and Iype(5) demonstrated histochemically the occurrence of lipase in the heart of the early chick embryo (40 hr). They also assessed quantitatively the changes in activity of the enzyme in this organ during development and showed that increase of lipase activity paralleled increase in heart rate. This lends further support to the view of Moog(6) that "the differentiation of the enzyme is directly related to the differentiation of function."

Liver is also known to be active during development. Bile secretion is known to appear about the 6th day of incubation(7). Needham(4) reviewing the older literature pointed out that fat metabolism is highest during the last week of incubation. We undertook the present study to find out precisely whether there is a direct relationship between this period of the chick embryo and the lipase activity in the liver.

Materials and methods. White Leghorn eggs of uniform size were used. They were incubated at 37.8 $\pm$ 0.01°C. After the desired period of incubation, the embryos were carefully removed from the yolk. The liver of embryos from 8th day to 19th day of incubation was used for quantitative determination of lipase activity. Up to the 10th day