TABLE II. Double Treatment with Estradiol, 50 μg/l, Followed by Testosterone, 50 μg/l, until Metamorphosis.

	Treatment	♂	♀	♀
A.	Control	55	—	—
	Estradiol (7 days)	—	1	17
	Estradiol followed by testosterone	16	5	—
B.	Control	72	—	63
	Estradiol (7 days)	1	5	18
	Estradiol followed by testosterone	7	4	10

slightly modified in the following experiment. Genetic males (ZZ), treated at the proper time with estradiol, become changed into females, if reared later in pure water. However, if the estradiol treatment is immediately followed by administration of testosterone, the animals revert back to males. Table II presents 2 experiments with identical procedures. But in the first one (A) the mother was a sex reversed male (ZZ) while in the second (B) she was of normal ZW constitution. Both experiments show that estradiol treatments feminize offspring of the ZZ type; but if it is immediately followed by testosterone, the reversion is wiped out, all ZZ individuals become males again (or intersexes). Under exactly the same conditions the ZW individuals develop normally into females. This abolition of initiated sex reversal is the only indication that androgens may inhibit the cortical inductors in a similar though much less effective way as estrogens are suppressing the medullary system.

Conclusions and summary. Continuation of experiments on sex determination, particularly with Xenopus, led to the conclusion that the primary inductors, corticin (of follicle cells) and medullarin (of interstitial cells) are antigens. The corresponding *anti-corticin* and *anti-medullarin,* suppressing the contrary inductor system, are responsible for the unisexual (gonochoristic) development of each individual. Normally the decision of cortical (female) or medullary (male) prevalence depends on the quantitative relationship between sex determining genes. Experimental reversal of this influence does not produce, or depend on, any perceptible temporary or permanent change in gene composition or function. If administered at a short critical period of sex determination (St. 26/27), estradiol causes ZZ larvae to develop permanently as females. This effect is brought about by depression of the medullary inductor system. The possibly ensuing development of hermaphrodite glands indicates that selectively the capacity of antibody (anticorticin) formation is more generally affected than that of the antigen (medullarin).

1. Witschi, E., *A. mikr. Anat.,* 1914, v86, 1.
2. Witschi, E., and Allison, J., *Anat. Rec.,* 1950, v108, 589.
3. Witschi, E., *A. Anat. micr. et Morph. exp.,* 1950, v39, 215.
4. Chang, C. Y., and Witschi, E., PROC. SOC. EXP BIOL. AND MED., 1955, v89, 150.
5. Gallien, L., *C. rend. Acad. Sci.,* 1953, v237, 1565.
6. Chang, C. Y., *J. Exp. Zool.,* 1953, v123, 1.
7. Witschi, E., *Development of Vetebrates,* Saunders, Philadelphia & London, (1956).
8. Stern, C., *Analysis of Development,* Saunders, Philadelphia and London, (1955), 151.
9. Delbrück, M., *Angew. Chem.,* 1954, v66, 391.

Received July 27, 1956. P.S.E.B.M., 1956, v93.

Differential Distribution of Enzyme Hydrolyzing Acetylsalicylic Acid (Aspirin) in Certain Fluids of the Rat.* (22689)

HARRY BECKMAN.
Department of Pharmacology, Marquette University School of Medicine, Milwaukee.

It appears likely that degradation of salicylic acid occurs principally, though probably not exclusively, in the kidneys since metabolites appearing in urine are not recoverable from the blood(1). The process is probably a complex one involving filtration at

* Partially supported by a grant from the Wisconsin Heart Assn.

the glomerulus, tubular reabsorption, tubular conjugation and tubular excretion. However, the conversion of acetylsalicylic acid (hereinafter referred to simply as aspirin) into salicylic and acetic acids apparently takes place widely throughout the body(2), and it therefore becomes desirable to learn whether there is any differential distribution in various body fluids of the enzyme that catalyzes this hydrolysis. The object of the present study was to determine whether there are differences in rates of *in vitro* production of salicylic acid when aspirin is incubated in presumable immediate fluids of contact after gastrointestinal absorption in the rat (*i.e.*, abdominal thoracic duct lymph and portal vein blood) as contrasted with the systemically circulating blood.

Methods. Obtaining the fluids. Male Sprague-Dawley rats weighing between 175 and 225 g and full-fed on our usual adequate laboratory diet until the morning of the experiments, were used in 3 groups. In the group in which lymph was to be diverted for collection outside the body the animals were anesthetized with ether and the thoracic duct cannulated by the method of Bollman *et al.* (3). This technic comprises entrance into abdominal cavity through a large left flank incision, puncture of thoracic duct at approximately 1.5 cm above the receptaculum chyli, tying in a polyethylene tube (O.D. 0.062 cm, I.D. 0.045 cm) for drainage of lymph, and bringing the tube out of the cavity through puncture wound in midline of abdomen. On completing the silk-suture closure both the left flank wound site and that of the stab are painted over with collodion. The anesthetic is then withdrawn and lymph collection begins with animal confined in the Bollman cage(4), in which considerable freedom of movement is allowed without permitting claws or teeth to reach wound or protruding tube. Lymph was collected during 6 hours in a small centrifuge tube into which was placed at the beginning one small drop from a No. 27 needle of heparin sodium solution (10,000 units/ml) and a similar drop added as each ml of lymph accumulated. The collection tube was continuously twirled in beaker of ice water to insure that enzyme activity stopped at the moment a drop of lymph entered it and refrigerated at 4°C at end of sixth hour. In all instances, with 2 or 3 animals producing simultaneously, a lymph pool adequate for preparation of the aspirin solution was obtained through collections on 2 or 3 consecutive days. Animals bled from portal vein were anesthetized with ether, the abdomen opened in midline from manubrium sterni to symphysis pubis, the gastrointestinal structures displaced to gain access to the portal vein, the latter tied off close to point of entrance into the liver, and 2 ml of blood collected through syringe and needle, the latter containing 2 small drops of heparin solution, by direct puncture of the vein. The individual blood specimens were quickly cooled by twirling in beaker of ice water and a sufficient number accumulated to supply a plasma pool of equal size to that of lymph. The plasma obtained by centrifugation at 3500 r.p.m. for 10 minutes was stored in refrigerator. The blood specimens that were to represent systemically circulating blood were obtained by direct heart puncture under ether anesthesia of the third group of rats, cooling the 2 ml heparinized specimens as in the case of portal vein bloods and refrigerating the pooled plasma. *Determination of enzymatic hydrolysis.* On second or third day the 3 separate pools (lymph, portal vein plasma and heart's blood plasma) were filtered and 10 ml of each were placed in a separate flask into which aspirin had been previously introduced. The flasks were shaken vigorously for 2 minutes, then incubated at 38°C together with a flask containing only water and aspirin. The concentration of aspirin in all flasks was 50 mg/100 ml. At precisely 20, 40, 60, 80, 100 and 120 minutes after beginning of incubation, 1 cc aliquots were withdrawn from the flasks and the salicylic acid content determined by the method of Brodie *et al.*(5), in which the salicylic acid is extracted in acidulated ethylene dichloride, transferred to the water phase, an iron reagent added to give color, and read in the spectrophotometer at 540 millimicrons.

Results. The recoveries of salicylic acid from the 3 fluids are shown in Fig. 1, in which the data are charted as percentage recovered

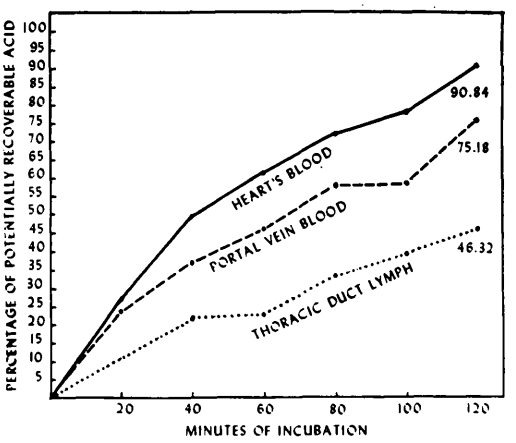

FIG. 1. Percentage recovered of potentially recoverable salicylic acid after *in vitro* incubation of acetylsalicylic acid in the respective fluids (rat).

of the potentially recoverable acid, using the means of 4 separate pools of each fluid at each of the time intervals. The highest recoveries at all intervals were from heart's blood, next highest from portal vein blood, and lowest from lymph. Throughout the entire 2-hour period the hydrolysis of aspirin proceeded at only about one-half the rate in lymph as in heart's blood. It is doubtful if the findings in the portal vein blood are truly representative of the enzymatic-hydrolysis-potential of that fluid directly as it leaves the site of principal aspirin absorption in the small intestine, because the specimens were taken without tying off the confluent veins from the stomach, spleen and pancreas, and therefore they contain some blood that had recently traversed the organs named. The presently designated portal vein blood is therefore actually such blood as it is delivered to the liver but it is not truly the first sanguine fluid that is encountered as aspirin is absorbed.

Discussion. The data establish that aspirin is hydrolyzed much less rapidly in lymph than in blood. It will be the objective of subsequent studies to determine whether the respective rates can be influenced by any adjuvant measures in the hope that the findings may help in the development of a satisfactory technic for determining salicylate antiphlogistic action in the laboratory.

Summary. The *in vitro* enzymatic hydrolysis of acetylsalicylic acid (aspirin) has been found to proceed only about one-half as fast in the abdominal thoracic duct lymph of the rat as in the heart's blood of the same animal.

1. Gutman, A. B., Yü, T. F., and Sirota, J. H., *J. Clin. Invest.*, 1955, v34, 711; Hoffman, W. S., and Nobe, C., *J. Lab. and Clin. Med.*, 1950, v35, 237.

2. Smith, P. K., *J. Pharmacol. and Exp. Therap.*, 1949, v97, 353.

3. Bollman, J. L., Cain, J. C., and Grindlay, J. H., *J. Lab. and Clin. Med.*, 1948, v33, 1349.

4. Bollman, J. L., *ibid.*, 1948, v33, 1348.

5. Brodie, B. B., Udenfriend, S., and Coburn, A. F., *J. Pharmacol. and Exp. Therap.*, 1944, v80, 114.

Received July 30, 1956. P.S.E.B.M., 1956, v93.

Two Types of γ-Globulin Differing in Carbohydrate Content. (22690)

H. J. MÜLLER-EBERHARD, HENRY G. KUNKEL AND EDWARD C. FRANKLIN
Rockefeller Institute for Medical Research, New York City.

Considerable evidence has accumulated suggesting that certain antibodies in human (1,2,3) as well as horse sera(4,5) are of higher molecular weight than other antibodies and the bulk of γ-globulin. A sedimentation constant of approximately 19 S has usually been observed in the ultracentrifuge indicating a molecular weight close to 1 million instead of the ordinary 150,000. The possibility has been raised that these large proteins are aggregates of the low molecular weight components(6). Recent experiments employing zone electrophoresis(7) have furnished evidence that normal human γ-globulin contains from 5-10% of 19 S material as a constant constituent. This is localized primarily in the faster migrating portion of the γ-globulin. Gamma globulin prepared by