1. Belding, H. S. and Hertig, B. A., J. Appl. Physiol. 17, 103–106 (1962).
2. Benzinger, T. H., Symp. Soc. Exptl. Biol. 18, 49-80 (1964).
3. Bullard, R. W., J. Appl. Physiol. 19, 137-141 (1964).
4. Bullard, R. W., Banerjee, M. R., and MacIntyre, B. A., Intern. J. Bioclimatol. Biometeorol. 11, 93-104 (1967).
5. Hardy, J. D. and Stolwijk, J. A. J., J. Appl. Physiol. 21, 1799-1806 (1966).
6. Issekutz, B., Jr., Hetenyi, G., Jr., and Diosy, A., Arch. Int. Pharmacodyn. 83, 133-142 (1950).
7. Janowitz, H. and Grossman, M. I., J. Invest. Dermatol. 14, 453-458 (1950).
8. Randall, W. C., Am. J. Physiol. 150, 365-371 (1947).
9. Randall, W. C., Deering, R., and Daugherty, I., J. Appl. Physiol. 1, 53-59 (1948).
10. Randall, W. C., Wurster, R. D., and McCook, R. D., and Brockhouse, J. E., Archiv. Environ. Health 11, 430-441 (1965).
11. Smith, P. E., Jr. and James, E. W., Arch. Environ. Health 9, 332-342 (1964).
12. Stolwijk, J. A. J. and Hardy, J. D., Arch. Ges. Physiol. 291, 129-162 (1966).
13. Stolwijk, J. A. J. and Hardy, J. D., J. Appl. Physiol. 21, 967-977 (1966).
14. Van Beaumont, W. and Bullard, R. W., Science 147, 1465-1467 (1965).
15. Van Beaumont, W., Bullard, R. W., and Banerjee, M. R., Dermatol. Dig. 5, 75-87 (1966).

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Sulfate Metabolism in the Intestinal Mucosa of Weanling and Adult Rats* (32825)

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The sulfate radical is physiologically and pharmacologically important to a variety of biological processes since it participates in the metabolism of many endogenous and exogenous substrates (1–3). Primarily, hydrolyzing (sulfatase) and transferring (sulfokinase) enzymes are involved. Studies which characterize these enzyme systems have been pursued principally in mammalian liver. However, sulfate metabolism also has been demonstrated in several other tissues, including the mucosa of the intestine of adult rats (4,5). This would permit orally administered compounds to undergo biotransformations involving sulfation or desulfation in the gastrointestinal tract. Such metabolic activity conceivably may play a very important role in detoxication and/or absorption processes involving facilitated or active transport.

The activities of several enzymes present in the mucosa of the intestine, their activity patterns during development, and their relationship to physiological events have been reported (6–8). Preliminary investigations in our laboratories demonstrated a difference in sulfate metabolism of immature vs adult rats. Peak activity in the intestine appeared to occur at 13 days of age with a progressive decline to adult values. The present study represents a further investigation of this phenomenon and an attempt to delineate the respective roles of sulfokinase and sulfatase enzymes in jejunal and ileal portions of the small intestine at two stages of development. Direct comparisons have been drawn between intestinal enzymic activity in suckling (13-day-old) and adult (43-day-old) rats.

Materials and Methods. Thirteen- and 43-day-old male and female albino Wistar rats were employed as experimental animals. Thirteen-day-old rats were suckled by their mothers and adults were maintained on regular Purina Lab Chow and water *ad libitum*. The animals were sacrificed by decapitation, the

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[1] On leave of absence from the Institute of Mother and Child Care, Prague, Czechoslovakia.

abdomen was opened, and polyethylene tubing was inserted into the duodenum. The intestine was flushed with 5 ml of ice-cold isotonic KCl and the duodenum was discarded. The remaining small intestine was divided into three sections of approximately equal length. Only the proximal (jejunal) and distal (ileal) segments were used experimentally. These intestinal segments were cut open and the mucosa was scraped off with a spatula. Tissues were kept at 4°C throughout these procedures. These tissue scrapings were immediately homogenized with a Potter homogenizer (plastic pestle) in one volume of ice-cold 0.15 *M* KCl solution. The homogenate was diluted with additional KCl solution such that 0.25 gm of tissue (wet wt.) were present in 1.0 ml of homogenate. With this concentration, measured enzymic activities were found to be directly proportional to the amount of homogenate added to incubation flasks.

Sulfokinase activity was measured according to a modification of the method of Percy and Yaffe (9). The method is based on the liberation of free *p*-nitrophenol (*p*-NP) after the transfer of sulfate to *m*-aminophenol (*m*-AP) which serves as the sulfate acceptor. Incubation flasks contained 2.67×10^{-2} *M* *m*-AP, 0.15 *M* Tris-HCl buffer (pH 6.0–9.0), 1.1×10^{-2} *M* ethylenediamino-tetraacetic acid, disodium salt, 1.8×10^{-3} *M* sodium adenosine diphosphate (crude preparation which contains 3′-phosphoadenosine-5′-phosphate (PAP), and 0.1 ml of 25% homogenate in a final volume of 3.0 ml. Flasks were preincubated for 5 min at 37°C. The substrate (*p*-nitrophenyl sulfate as the potassium salt in 2.67×10^{-2} *M* final concentration) was then added. Mixtures were incubated at 37°C for 15 min. Control incubation flasks contained all reagents except the sulfate acceptor (*m*-AP) and were handled in an identical fashion. After incubation, 2.0 ml of 95% ethanol was added to the flasks. The mixture was centrifuged and 2.0 ml of the clear supernatant were diluted with an equal volume of 1 *N* NaOH. The optical density (OD) of the alkaline solutions was read at 400 mμ on a Gilford 300 spectrophotometer. The difference between the OD's of the test solutions and the OD's of the corresponding controls was taken as a measure of the sulfokinase activity of these homogenates. The OD's of control solutions could also be utilized as one measure of the sulfatase activity of such homogenates. Transfer of the sulfate radical to *m*-AP was verified using labelled PAP^{35}S and paper chromatography procedures.

Sulfatase activity was measured according to a modification of the method of Roy (10). The incubation mixtures consisted of 0.1 *M* sodium acetate buffer (pH 3.5–6.0) or Tris-HCl buffer (pH 6.0–8.0), 2.5×10^{-3} *M* *p*-nitrocatechol sulfate, potassium salt, (*p*-NCS) and 0.1 ml of 25% tissue homogenate in a total volume of 1.0 ml. Incubation proceeded for 60 min at 37°C. Reactions were stopped by the addition of 5.0 ml of 1 *N* NaOH. The mixture was centrifuged and the OD's of the alkaline supernatants (containing the colored *p*-nitrocatechol) were determined spectrophotometrically at 515 mμ. For the reagent blank, the NaOH was added immediately prior to the addition of the substrate.

Concentrations of protein in the homogenates were determined according to the method of Lowry, *et al.* (11).

Results. The pH optimum for rat intestinal sulfatase activity is depicted in Fig. 1. Homogenates from the intestines of adult rats were used for these determinations and *p*-NCS was employed as substrate. For ileal and jejunal

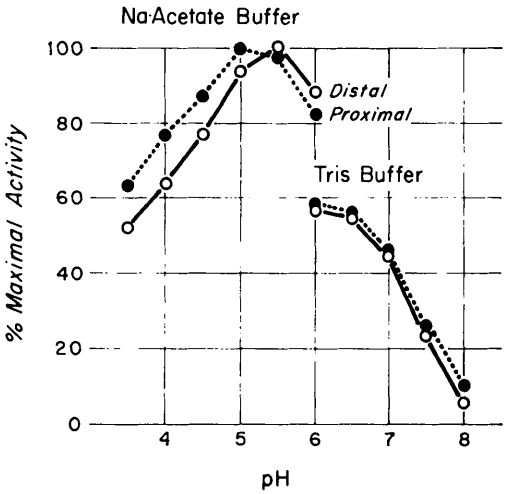

FIG. 1. The pH dependence of intestinal sulfatase activity. *p*-Nitrocatechol sulfate was employed as substrate.

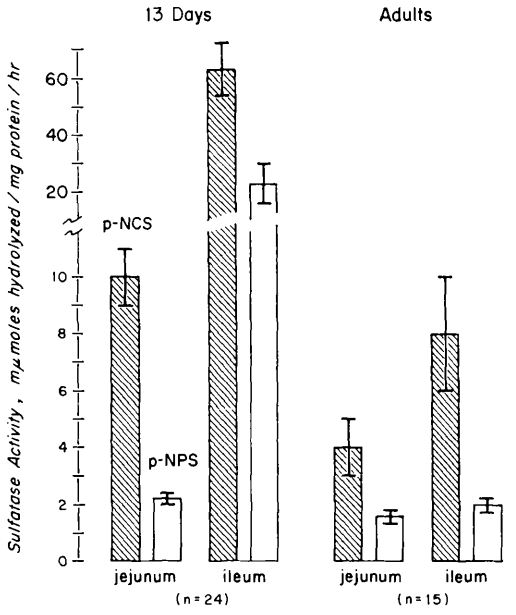

FIG. 2. Sulfatase activity of rat intestinal homogenates using *p*-nitrocatechol sulfate (*p*-NCS) and *p*-nitrophenyl sulfate (*p*-NPS) as substrates. Brackets represent standard errors. Numbers in parentheses represent the animals used in each experiment.

segments pH were essentially identical and maximal activity was observed with a pH range of 5.0–5.5.

The relative activities *in vitro* of ileal vs jejunal sulfatase activities in 13- and 43-day-old rats are shown in Fig. 2. It should be noted that sulfatase activity of the ileal segment is much higher than jejunal activity in suckling animals. No significant difference was observed between the segments in preparations from adult animals. This was noted when employing either *p*-NCS substrate at pH 5.0 or *p*-NPS as substrate at pH 7.8.

Figure 3 illustrates the determination of the pH optimum of intestinal sulfokinase activity for adult rats. As was the case with sulfatase, pH curves for ileal and jejunal segments were very similar. Using *m*-AP as the sulfate acceptor, the pH optimum appeared to be approximately 7.8 for both segments. The enzyme of the distal segment appeared to be somewhat more sensitive to pH changes, e.g., at pH 6.0 the activity of the distal segment was only 26% of that of pH 7.8. The activity of the proximal segment, however, was approximately 50% of maximal activity at pH 7.8.

Sulfokinase activity in the jejunum was significantly ($p<0.05$) higher than that observed in the ileum in both adults and suckling (Fig. 4). Ileal sulfokinase activity in 13-day-old rats was not detectable, owing perhaps to the very high sulfatase activity of this segment. However, jejunal sulfokinase activity was 3–4 times as high in the suckling as in the adult. Sulfatase activity was always much higher (3–5 ×) in the intestine of suckling rats when compared to adult rats. No sex differences could be observed in these experiments.

Discussion. These experiments illustrate marked differences in intestinal sulfate metabolism in suckling vs adult rats. The results also demonstrate large differences between jejunal and ileal segments with regard to their capacity to sulfate or desulfate the substrates employed. Such differences could be postulated to reflect variations in the physiologic role of the segments of the intestine during postnatal development. Teleologically, it is difficult to assign definitive reasons for such variations in enzymic activity, particularly when it is realized that the functional significance of this enzymic activity in the gastrointestinal tract is poorly understood. Sulfate metabolism could play an important role in transport processes, since sulfated compounds are more polar and

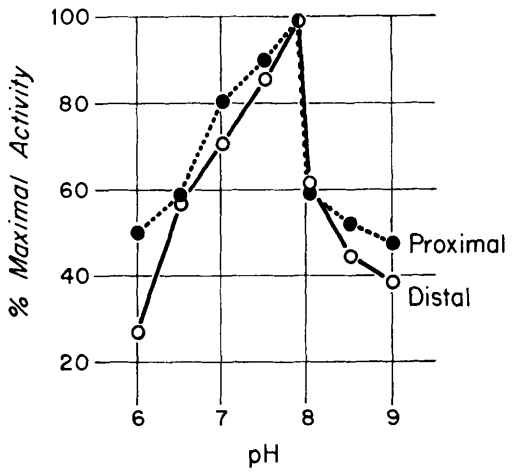

FIG. 3. The pH dependence of intestinal sulfokinase activity. *m*-Aminophenol was employed as sulfate acceptor and *p*-nitrophenyl sulfate as sulfate donor.

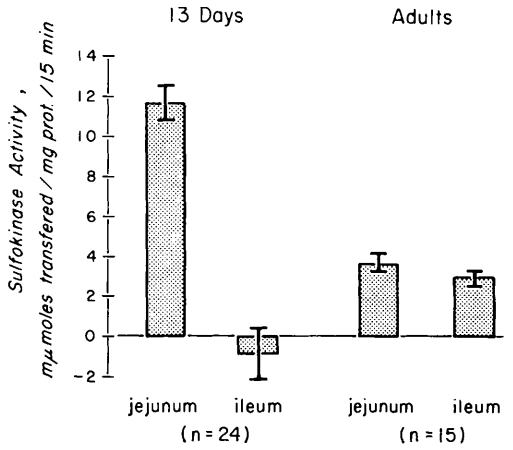

FIG. 4. Sulfokinase activity of rat intestinal homogenates. Brackets represent standard errors. Numbers in parentheses represent the animals used in each experiment. Sulfokinase activity (measured by mμ-moles of *p*-nitrophenol (*p*-NP) liberated) is corrected for *p*-NP liberated as a result of sulfatase activity.

would be transported across biological membranes with greater difficulty than nonsulfated compounds. According to this concept, increased sulfatase activity should facilitate transport, and increased sulfokinase activity should diminish transport of the endogenous or exogenous substances involved. The balance between sulfokinase and sulfatase activities then, would to some degree, determine the ease of transport of such compounds.

In mouse liver homogenates, activities of enzymes participating in sulfate metabolism were found to be highest during the suckling period (9). If the small intestine is considered as a unit (jejunum and ileum combined), a similar conclusion is obtained. The reason for such developmental changes is probably complex, and may include such factors as induction or stabilization by substrate, developmental changes in tissue function and cell type, altered sources of nutrients, the influences of hormones, and other environmental factors (12–14).

Studies of intestinal β-galactosidase (15), β-glucuronidase (7), peptidase (16), and alkaline phosphatase (17) have shown that ileal and jejunal segments display different activities with respect to these enzymes. This also appears to be true for intestinal sulfatase and sulfokinase enzymes. The significance of these apparently marked differences to the metabolism and transport of both xenobiotic and endogenous materials remains obscure and should be further investigated.

Summary. Sulfate metabolism in homogenates of rat intestinal mucosa was investigated in the jejunal and ileal segments. Direct comparisons were made between activities of homogenates of adult (43-day-old) and suckling (13-day-old) rats. The hydrolysis of *p*-nitrocatechol sulfate or *p*-nitrophenyl sulfate proceeded most rapidly in incubation mixtures which contained homogenates of ileal mucosa from 13-day-old rats. Sulfokinase activity was observed to be highest in homogenates of jejunal segments from suckling animals. In both cases the differences were marked, and may suggest important developmental changes in physiological function of these two segments of the gastrointestinal tract.

1. DeMeio, R. H. and Tkacz, L., J. Biol. Chem. **195**, 175 (1952).

2. Robbins, P. W. and Lipmann, F., J. Biol. Chem. **229**, 837 (1957).

3. Gregory, J. D. and Lipmann, F., J. Biol. Chem. **229**, 1081 (1957).

4. Hsu, L. and Tappel, A. L., Biochim. Biophys. Acta. **101**, 83 (1965).

5. Rose, Y. and Lipmann, F., J. Biol. Chem. **223**, 6 (1958).

6. Koldovsky, O., Heringova, A., Hoskova, J., Jirsova, V., Noack, R., Friedrich, and M., Schenk, G., Biol. Neonatorum **9**, 33 (1965).

7. Heringova, A., Jirsova, V., and Koldovsky, O., Can. J. Biochem. Physiol. **43**, 173 (1965).

8. Jirsova, V., Koldovsky, O., Heringova, V., and Hoskova, J., Biol Neonatorum **8**, 30 (1965).

9. Percy, A. K. and Yaffe, S. J., Pediatrics **33**, 965 (1964).

10. Roy, A. B., Biochem. J. **59**, 8 (1955).

11. Lowry, O., Rosenbrough, N. J., Farr, A. L., and Randall, J., J. Biol. Chem. **193**, 265 (1951).

12. Dawkins, M. J. R., Brit. Med. Bull. **22**, 31 (1966).

13. Augustinsson, K. B. and Henricson, B., Acta Physiol. Scand. **64**, 33 (1965).

14. Lewis, A. A. M. and Hunter, R. L., J. Histochem. Cytochem. **14**, 418 (1966).

15. Koldovsky, O. and Chytil, F., Biochem. J. **94**, 266 (1965).

16. Noack, R., Koldovsky, O., Friedrich, M.,

Schenk, G., Heringova, A., and Jirsova, V., Biochem. J. 96, 378 (1966).

17. Pelichova, H., Koldovsky, O., Heringova, A., Jirsova, V., and Kraml, J., Canad. J. Biochem. Physiol., in press.

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Immune Response Following Simultaneous Administration of Attenuated Trivalent Poliovirus and Type 4 Adenovirus Vaccines* (32826)

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The efficacy of a live oral adenovirus type 4 vaccine in controlling acute respiratory disease due to this agent in military recruits is well established (1,2). Since adenovirus infections occur early in training and cause a costly interruption of the training cycle, this vaccine should be administered shortly after arrival at a recruit camp (3). Marine recruits at Parris Island, South Carolina currently receive seven separate immunizations, during their first training week, including oral trivalent poliovirus, and smallpox vaccines.

The question of diminished immune response following simultaneous administration of live virus vaccines has not been completely resolved. The Public Health Service Advisory Committee on Immunizations suggests that whenever feasible attenuated poliovirus and measles virus vaccines should be administered separately with at least 1 month between immunizations (4). Other reports, however, indicate that attenuated measles vaccine and smallpox vaccine may be simultaneously administered (5). Serologic and epidemiologic evidence of interference by enteric viruses with attenuated poliovirus vaccination is well documented (6–10). Attenuated adenovirus type 4 immunizes by establishing a silent infection in the lower gastrointestinal tract but interference of this infection by enteric viruses has not been reported. In 1966 successful simultaneous immunization with monovalent type 1 attenuated poliovirus and the oral live adenovirus type 4 vaccine was achieved (11). This paper describes the results of simultaneous administration of live attenuated poliovirus and adenovirus vaccines; no serologic evidence of interference was noted.

Methods. Three platoons of recruit volunteers at Marine Corps Recruit Training Depot, Parris Island, South Carolina were vaccinated simultaneously according to the following schedule: (a) Platoon 240 received live attenuated adenovirus type 4 as a tablet containing $10^{5.3}$ $TCID_{50}$.[1] (b) Platoon 246 received a single dose of the attenuated trivalent poliovirus vaccine.[2] (c) Platoon 247 was vaccinated simultaneously with both the attenuated poliovirus and adenovirus vaccines.

All recruits were bled immediately prior to vaccination and 21 days later. Sera were stored at —20°C before use. Serum neutralizing antibody titers to adenovirus type 4 were determined in human embryonic kidney tissue culture employing 10–32 $TCID_{50}$ adenovirus type 4. Prevaccination sera from platoons 240 and 247 were screened at a 1:4 dilution for adenovirus type 4 neutralizing antibody. Quantitative antibody response to the attenuated adenovirus vaccine was determined on sera from 25 recruits lacking initial neutralizing antibody at the 1:4 dilution.

Poliovirus neutralizing antibody titers were determined on pre- and postvaccination sera

* The opinions or assertions contained herein are the private ones of the writers and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

[1] The experimental vaccine (Lot No. 16CI-00101) was prepared by Wyeth Laboratories. This was supported by Public Health Service Contracts 43-62-842 and 43-62-40 from the Vaccine Development Branch, National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda, Maryland.

[2] Orimune (Lederle) containing $10^{5.9}$, $10^{5.0}$, and $10^{5.7}$ $TCID_{50}$ Types I, II, and III poliovirus, respectively.