Stimulation of *in Vitro* Renal Organic Acid and Base Transport by Rabbit Serum[1] (36607)

G. H. HIRSCH, J. E. ECKER, AND J. B. HOOK
(Introduced by W. J. Johnson)

Pharmacology Section, Health Protection Branch, Ottawa, Ontario, K1A OL2 Canada, and Department of Pharmacology, Michigan State University, East Lansing, Michigan 48823

Recent studies have described the developmental pattern of the anionic transport system in rabbits as measured by the ability of renal cortical slices to accumulate *p*-aminohippurate (PAH) (1, 2). Treatment of young rabbits with substrates of the anionic transport system increased activity, and enhanced maturation, of the PAH transport system (2, 3). These investigations also demonstated that PAH accumulation by renal cortical slices from rabbit fetuses was higher than in cortical slices prepared from one day old rabbits (3). It was suggested that the greater accumulation of PAH by fetal cortical slices was associated with the sudden increase in functional load and the changes in serum content that occur in the kidney at birth.

Experiments utilizing rat serum have demonstrated that low concentrations of normal serum stimulate PAH accumulation by rat kidney cortical slices, while azotemic serum, or high concentrations of normal serum, have an inhibitory effect (4–6). The effects produced by azotemic and normal rat or human serum on hippurate transport have been attributed to the presence of dialyzable substances which competitively inhibit organic anion transport (6, 7).

These observations suggest that serum composition has significant effects on renal transport activity. The objectives of the present study were to determine the effects of serum from rabbits at various ages on PAH and *N*-methylnicotinamide (NMN) accumulation by rat and rabbit kidney slices, and to partially characterize the serum factors responsible for these effects.

Materials and Methods. New Zealand white rabbits were bred in the departmental animal quarters, and the offspring were left with their mothers until used for experimentation. Rabbits were stunned by a blow on the head, and after severing the carotid arteries, the blood was collected and allowed to clot. Fetal rabbit serum was obtained from fetuses procured surgically by caesarean section one day before scheduled delivery. The serum obtained after centrifugation was used immediately or stored at —4°.

Ultrafiltrates of rabbit serum were obtained by passing serum through Millipore filters having an average pore size of 0.22 μ. Dialysis of 2 ml serum aliquots was performed in Visking tubing for 18 hr against 1 liter of constantly stirred 0.1 *M* phosphate buffer at 4°.

Male Sprague–Dawley or Wistar rats (225–300 g) were killed by cervical dislocation; the kidneys were removed immediately, weighed, and placed in cold saline. Renal cortical slices from several rats were pooled in each experiment and about 100 mg of tissue was added to beakers containing Cross and Taggart medium (8) which included 7.4 $\times$ 10^{-5} *M* PAH and 6 $\times$ 10^{-6} *M* NMN–C^{14} (4.6 m Ci/mmole). Rabbit serum was added to each beaker in the amounts of 0.25, 0.5 or 1.0 ml, to give a total volume of 3.0 ml (corresponding to serum dilutions of 1:12, 1:6 and 1:3, respectively). Similar amounts of blank medium (no PAH or NMN) were added to control beakers. All incubations were carried out at 25° for 90 min under a gas phase of 100% oxygen. Accumulation of PAH and NMN by renal cortical slices was

[1] Supported in part by U.S. Public Health Service grant 10913.

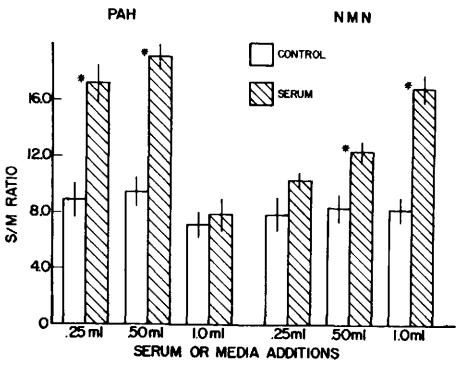

FIG. 1. Effect of increasing amounts of serum from 4 wk old rabbits on PAH and NMN accumulation by rat kidney slices. Each bar represents the mean (±S.E.) of five experiments. The asterisks indicate those values which are significantly different from their respective controls ($p < .05$).

studied using the methods described by Cross and Taggart (8) and Hook and Munro (4). Results were expressed as the slice/medium (S/M) ratio, where S equals milligrams per gram of tissue (wet weight) or disintegrations per minute per gram of tissue, and M equals milligrams per milliliter of medium or disintegrations per minute per milliliter of medium. PAH was estimated by the method described by Smith *et al.* (9) while NMN–C^{14} was counted in a scintillation counter as described by Hirsch and Hook (2).

The data obtained were analyzed statistically by Student's *t* test, group comparison (10). The 0.05 level of probability was used as the criterion of significance.

Results. Serum from 4 week old rabbits caused a significant enhancement of both PAH and NMN accumulation by rat kidney slices (Fig. 1). Essentially maximum stimulation of PAH transport was produced upon the addition of 0.25 ml of serum, while no stimulation was obtained with 1.0 ml serum. In contrast, NMN accumulation by the rat kidney slices was enhanced in a "dose dependent" manner.

The addition of 0.5 ml serum from rabbits ranging in age from —1 day (fetuses) to adults resulted in stimulation of both PAH and NMN accumulation that was of comparable magnitude at all ages studies except one (Table I). The only exception was after the addition of serum from fetal rabbits, where the enhancement of PAH uptake was greater than at the other ages.

Ultrafiltrates of serum from adult rabbits retained their ability to stimulate both PAH and NMN uptake by rat kidney cortical slices (Fig. 2). Enhancement of PAH accumulation was produced by serum or serum ultrafiltrates only when 1:12 or 1:6 dilutions were used, while enhanced NMN accumulation was produced at all the serum concentrations studied. Dialysis of adult rabbit serum resulted in a complete loss of its capacity to stimulate both PAH and NMN renal uptake, at all three serum concentrations (Fig. 3).

When serum from adult rabbits was added to incubation medium containing adult rabbit kidney cortical slices, stimulation of the PAH SM ratio, but not the NMN S/M

TABLE I. Effect of Serum from Rabbits of Varying Ages on PAH–NMN Uptake (S/M) by Rat Kidney Slices.[a]

Age	PAH S/M		NMN S/M	
	Control	Serum	Control	Serum
—1 day	9.32 ± 0.74	28.53 ± 2.72[b]	7.29 ± 0.25	14.66 ± 1.32[b]
1 day	8.49 ± 0.93	14.96 ± 1.37[b]	7.35 ± 0.17	14.46 ± 0.68[b]
1, 2 wk	9.16 ± 0.56	17.72 ± 2.68[b]	6.93 ± 0.82	12.48 ± 0.08[b]
4 wk	9.47 ± 1.18	18.98 ± 0.90[b]	8.37 ± 1.15	12.34 ± 0.54[b]
Adult	8.79 ± 0.86	15.11 ± 1.37[b]	5.53 ± 0.67	13.60 ± 1.30[b]

[a] In each case, 0.5 ml rabbit serum was added to beakers containing 2.5 ml medium and renal cortical slices from adult male rats, while blank media was added to control beakers. Each value is the mean (± SE) obtained in 3 to 5 experiments.

[b] Significantly different from control ($p < .05$).

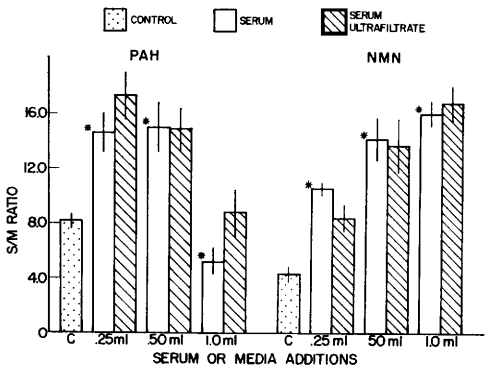

FIG. 2. Effect of ultrafiltration of adult rabbit serum on PAH and NMN accumulation by rat kidney slices. Each represents the mean (±S.E.) of six experiments. The asterisks indicate those values significantly different from their respective controls ($p < .05$). None of the values obtained using rabbit serum were significantly different than the values obtained using serum ultrafiltrate ($p < .05$).

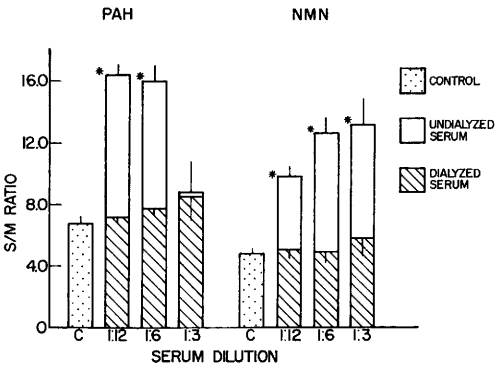

FIG. 3. Effect of dialysis of adult rabbit serum on PAH and NMN accumulation by rat kidney slices. Each bar represents the mean (±S.E.) of nine experiments. The asterisks indicate the undialyzed serum values that are significantly different from their respective controls ($p < .05$). None of the dialyzed serum values were significantly different from their respective controls ($p < .05$).

ratio, was observed (Table II). Essentially maximum stimulation of PAH uptake was obtained using 0.25 ml serum, similar to the results for rat kidney slices shown in Fig. 1.

Discussion. Rabbit serum contains substances which facilitate both organic anion and organic cation accumulation by rat renal cortical slices. These serum factors are low molecular weight substances, since they are both dialyzable and ultrafilterable. The observation that both PAH and NMN accumulation by rat kidney slices is stimulated by rabbit serum is of particular interest. The renal cationic transport system is poorly developed in rabbits (11) and its activity is not altered by compounds which enhance anionic transport activity (2). The low activity of the cationic transport system in rabbits is emphasized by the finding in the present study that rabbit serum is able to enhance PAH, but not NMN, accumulation by rabbit kidney slices. This compares with the results of Bourke *et al.* (12), who found that rat serum did not alter renal base transport by isolated rabbit renal tubules.

A number of organic anions involved in intermediary metabolism such as lactate, pyruvate and acetate have been shown to stimulate, and in some cases inhibit, hippurate transport in kidney slices, depending on the concentration used (8, 13). At the present time it is not possible to determine the relative importance of these metabolizable anions versus the role of nonmetabolized anions such as hippurate in altering renal anionic transport. Since organic anion transport may

TABLE II. Effect of Rabbit Serum on PAH–NMN Uptake by Rabbit Kidney Slices.[a]

		Serum addition		
	Control	.25 ml	.50 ml	1.0 ml
PAH S/M	13.02 ± 2.56	25.93 ± 2.17[b]	28.03 ± 3.76[b]	27.70 ± 2.49[b]
NMN S/M	2.14 ± 0.06	2.32 ± 0.19	2.19 ± 0.12	2.25 ± 0.23

[a] Serum from adult male or female rabbits, or blank medium for controls, was added to beakers containing PAH–NMN medium and renal cortical slices from adult rabbits. Each value is the mean (± SE) from five experiments.

[b] Significantly different from control, $p < .05$.

be a means of transport of major energy substrates of the renal cortex, (14), the stimulatory effect of rabbit serum on PAH accumulation by rat kidney slices may be associated with increased availablilty of these substrates. Oringer *et al.* (6) demonstrated that the rat serum factors which enhanced renal anionic transport were removed by passage through anion exchange columns, in addition to being dialyzable and ultrafilterable, indicating that these factors were low molecular weight anionic substances. The concomitant stimulation of organic acid and base transport by rabbit serum in this study suggests that other factors may also be involved. Preliminary studies demonstrated that protein binding was not important since recoveries of PAH and NMN averaged about 100% in all experiments, and S/M ratios of 1 were obtained when incubations were carried out under nitrogen. It is also unlikely that the addition of rabbit serum to the incubation beakers changed the electrolyte concentrations of the medium enough to alter PAH or NMN transport.

The ability of fetal rabbit serum to stimulate PAH accumulation by rat kidney slices to a greater extent than that produced by newborn rabbits is consistent with the observation that renal cortical slices from fetal kidneys generate higher PAH S/M ratios than slices from 1 day old rabbits (3, 15). The differences between azotemic and normal serum in their effects on anionic transport are due to differences in the quantity and quality of organic anions present in each (6), and it is possible that analogous differences exist between fetal and newborn rabbit serum. The stimulatory activity of serum from rabbits at 1–4 weeks of age was similar in the present study, suggesting that the marked developmental increase of *in vitro* anionic transport activity seen at 2–4 weeks in rabbits (2) is not associated with changes in serum composition.

Summary. Addition of rabbit serum to PAH–NMN medium (1:6 or 1:12 dilution) enhanced the accumulation of both the organic acid PAH and the organic base NMN by adult rat kidney cortical slices. Addition of rabbit serum to PAH–NMN medium containing rabbit kidney slices stimulated PAH uptake only. Adult rabbit serum retained its stimulatory effect on PAH–NMN accumulation by rat kidney slices after ultrafiltration of the serum, but the stimulatory effect was lost after dialysis, suggesting that low molecular weight substances were responsible for the observed results. These findings suggest that serum composition influences renal organic ion transport. The magnitude and specificity of serum-induced changes in PAH–NMN transport can provide information about the mechanisms involved in renal organic ion transport.

1. Hirsch, G. H., and Hook, J. B., Science **165**, 909 (1969).

2. Hirsch, G. H., and Hook, J. B., J. Pharmacol. Exp. Ther. **171**, 103 (1970).

3. Hirsch, G. H., and Hook, J. B., Can. J. Physiol. Pharmacol. **48**, 550 (1970).

4. Hook, J. B., and Munro, J. R., Proc. Soc. Exp. Biol. Med. **127**, 289 (1968).

5. White, A. G., Proc. Soc. Exp. Biol. Med. **123**, 309 (1966).

6. Orringer, E. P., Weiss, F. R., and Preuss, H. G., Clin. Sci. **40**, 159 (1971).

7. Preuss, H. G., Massry, S. G., Maher, J. F., Gilliece, M., and Schreiner, G. E., Nephron **3**, 265 (1966).

8. Cross, R. J., and Taggart, J. V., Amer. J. Physiol. **161**, 181 (1950).

9. Smith, H. W., Finkelstein, N., Aliminosa, L., Crawford, B., and Graber, M., J. Clin. Invest. **24**, 388 (1945).

10. Lewis, A. E., "Biostatistics." Reinhold, New York (1966).

11. McIsaac, R. J., J. Pharmacol. Exp. Ther. **168**, 6 (1969).

12. Bourke, E., Frindt, G., Preuss, H., Rose, E., Weksler, M., and Schreiner, G. E., Clin. Sci. **38**, 41 (1970).

13. Koishi, T., Jap. J. Pharmacol. **8**, 101 (1959).

14. Selleck, B. H., and Cohen, J. J., Amer. J. Physiol. **208**, 24 (1965).

15. Rennick, B., Hamilton, B., and Evans, R., Amer. J. Physiol. **201**, 743 (1961).

Received Mar. 28, 1972. P.S.E.B.M., 1972, Vol. 140.