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Albert H. Banner
Satoshi Sasaki
Philip Helfrich
Charles B. Alender
Paul J. Scheuer*

Hawaii Marine Laboratory,
University of Hawaii,
Honolulu 14, Hawaii.

* Department of Chemistry, University of Hawaii.

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Properties of Vessel Muscle Proteins extracted with Water or Salt Solutions of Low Ionic Strength

Earlier investigations on the relations of the electrolyte content of organs to hypertension in the rat have shown that only the increase of the potassium content of the vessel wall is characteristic of hypertension[1]. In the carotic strip of cow *in vitro*, potassium produces a rise in tension which lasts so long as the potassium concentration of the bath solution is not altered. Simultaneously with the penetration of potassium into the muscle fibre a rise in tension[2], viscosity and dynamic elasticity modulus[3] occurs. It may be concluded from this that the potassium which entered the muscle fibre discharges certain proteins and so increases the mechanical tension.

Starting from this hypothesis, I have made extracts of relaxed vessel muscles using water or salt solutions of ionic strength either lower than that in the intracellular fluid or equal to it. The muscle of the relaxed cow carotis was separated from other tissues. Then it was minced in a small meat grinder, mixed in a mortar for 10 min. at room temperature with 1·5 c.c./gm. of the chosen solution and finally centrifuged for 15 min. at 22,000*g*. Extracts so obtained have a high viscosity (η rel. = 6·02 at a protein concentration of 1·55 per cent; extract of striated muscle of the same animal η rel. = 1·61 at a protein concentration of 1·67 per cent) and show double refraction of flow. After addition of 0·6 *M* potassium chloride (*p*H 7·0) at 15–18° C., they turn into a more or less thixotropic gel after a few seconds. The gelling also takes place, but more slowly, at lower potassium concentrations so long as these exceed that in the intracellular space. Extracts prepared by the same procedure from striated muscle of the cow, rabbit or rat and from smooth muscle of the non-pregnant uterus, bladder and small intestine of the rabbit or the small intestine of the rat do not show the above-mentioned properties. (Extracts of smooth muscle of the bladder and small intestine of the cow show double refraction of flow.) This can be taken as a hint that the vessel muscle contains, besides actomyosin, other structure proteins which are responsible for the permanent tension. We have, therefore, in collaboration with Hamoir, investigated extensively vessel muscle extracts by electrophoresis and ultracentrifugation. The results of these investigations will be reported in another communication.

Our findings clearly demonstrate the presence of two structure proteins. The study of their solubility has enabled us to isolate these proteins and to investigate them separately, as well as the actomyosin which was isolated from the vessel muscle.

L. Laszt

Department of Physiology,
University of Fribourg,
Fribourg, Switzerland.

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Conjugation of *p*-Aminohippurate by the Kidney and Effective Renal Plasma-flow

The clearance of *p*-aminohippurate has become the standard method for the determination of effective renal plasma-flow, with the assumption that there is no conjugation of *p*-aminohippurate during its passage through the kidney from the blood to the urine. The metabolism of this substance in the body is considered to be similar to that of sulphanilamide which it resembles, namely, acetylation of the *para* amino group. *p*-Acetylaminohippurate and *p*-aminohippurate have similar clearances in the dog and man, so the former may be expected to depress maximal tubular excretion of the latter, but as neither *p*-acetylaminohippurate nor any metabolite in which the *para* amino group is substituted is estimated by the technique used for *p*-aminohippurate, conjugation at sites other than in the kidney should not affect the clearance of the latter. With sulphanilamide, conjugation occurred only in the liver of rabbits and rats, in the liver and spleen in cats, and not at all in the dog (cf. ref. 1). *p*-Aminohippurate itself was not conjugated by rabbit[2] or dog[3] kidney slices *in vitro*, although some conjugation was found with rat[4] and guinea pig[2] kidney slices. From the evidence available, Smith[1] came to the conclusion that conjugation in the kidney did not occur to any significant extent in the species studied (rat, rabbit, cat, dog and man), although it has been suggested by others that conjugation could explain low clearances of *p*-aminohippurate with falling plasma concentrations in man[5].

It will be seen from Table 1 that the conjugation of *p*-aminohippurate does occur to an appreciable extent in the kidney of a number of species; 95 ± 1·3 per cent (22 observations) was recovered after mild acid hydrolysis (100° C. for 30 min., final acid concentration 0·56 *N*). The findings in the rabbit agree with earlier reports, but in the cat only low rates were found in the liver and none demonstrable in the spleen. That this conjugation did interfere significantly with the determination of effective renal plasma-flow was shown in the guinea pig, in which the clearance of free + conjugated *p*-aminohippurate was 1·19 ± 0·05 ($P < 0·01$) (9 determinations) times that of free *p*-aminohippurate on the same samples, with a mean plasma concentration of 0·10 ± 0·015 μmole free *p*-aminohippurate/ml. At this concentration, the calculated values for conjugation and excretion give a value of 1·17 for the above ratio, if the clearance of *p*-aminohippurate is taken to be 13 ml./min. (the mean value of the 9 determinations) and the kidneys weigh 4 gm. each.

The rate of conjugation *in vitro* was maximal at 0·1 μmole/ml., so less error would probably be introduced at higher plasma concentrations, although