1.2 g/100 ml NaCl with 25 ml of a mixed buffer composed of 72 ml of $M/15$ $Na_2HPO_4$ and 28 ml of $M/15$ $KH_2PO_4$. The pH was 7.0 at 25°C. All samples of S protein were analysed for nitrogen by micro-Kjeldahl method; thereafter, they were brought to the same nitrogen content (mg 130%). The presence of haemoglobin in the solution of S protein should not interfere with the present results, since haemoglobin, as established before, does not cause haemagglutination.

The red blood cells were washed, before using, five times with 0.9% NaCl solution and made up to a 3% suspension in NaCl-buffer. The reaction was carried out on microscope slides. A large drop of cell suspension was added to each drop of S protein. Complete mixing was secured first by stirring, later by rocking to and fro. The progress of reaction was observed against a white background. The test is observed for 15, or at the most 30 min. Fine agglutinates were graded as one plus, medium-size as two plus, and large clumps as three plus.

As shown in Table I, S protein was active in producing agglutination of rabbit, rat and guinea-pig erythrocytes. However, in contrast to the above-mentioned studies, this haemagglutinating activity was not confined to the three rodent species here considered but was also found in the samples from horse blood. Moreover, guinea-pig S protein acted only upon the red cells of the same species whereas the human one failed to cause agglutination of cells of any species.

The explanation of these findings is not easy; it seems, however, reasonable to regard them as really due to S protein and not due to heterogenetic antibodies derived from residual plasma in the protein. Table II indicates, in fact, that there was no relationship between its haemagglutinating property and the cross reactions observed.

Table I. Haemagglutinating activity of S protein derived from man, horse, rabbit, guinea-pig and rat on the corresponding red cells

| Red cells from: | S protein from red cells of: | | | | |
|---|---|---|---|---|---|
| | Man[a] | Horse | Rabbit | Guinea-pig | Rat |
| Man | 0 | 0 | 0 | 0 | 0 |
| Horse | 0 | 0 | 0 | 0 | 0 |
| Rabbit | 0 | ++ | 0 | 0 | 0 |
| Guinea-pig | 0 | ++ | ++ | +++ | +++ |
| Rat | 0 | +++ | 0 | 0 | 0 |

[a] Human S protein was obtained from 0 Rh positive red cells of normal donors.

Table II. The lack of relationship between red cell agglutination by S protein and heterogenetic antibodies derived from residual plasma proteins

| Red cells from: | Plasma of: | | | | |
|---|---|---|---|---|---|
| | Man | Horse | Rabbit | Guinea-pig | Rat |
| Man | 0 | 0 | +++ | 0 | 0 |
| Horse | 0 | 0 | 0 | 0 | 0 |
| Rabbit | +++ | 0 | 0 | + | 0 |
| Guinea-pig | +++ | 0 | ++ | 0 | 0 |
| Rat | +++ | 0 | ++ | +++ | 0 |

*Riassunto.* La proteina «S» dello stroma eritrocitario di alcune specie animali (uomo, cavallo, coniglio, cavia, ratto) si è dimostrata capace, per alcune di esse, di agglutinare le emazie omologhe ed eterologhe. Il fenomeno sembra legato alla proteina «S» e non ad anticorpi eterogenetici derivanti da tracce di plasma presenti nella proteina stromale.

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## Androgen Influence on Protein and Enzyme Pattern of Rat Kidney[1]

Column chromatography on substituted cellulose ion exchanger has been used in previous researches to fractionate soluble proteins extracted from the submaxillary gland of male and of female mouse. Clear-cut differences in the protein composition of male and female glands were demonstrated, the chromatographic patterns appearing distinctive and characteristic for each sex[2].

By using column chromatography BOND was able to demonstrate a sex-specific protein, of still unknown function, in rat liver[3]. This protein is present in the male rat liver and is susceptible to hormonal manipulation. We have extended this type of investigation to other organs known for their sexual dimorphism. The present paper is concerned with chromatographic fractionation of proteins of extracts of male and female rat kidneys; four enzyme activities have been localized in the fractions; in addition the effect of testosterone treatment on the female pattern has been studied.

*Materials and Methods.* Adult albino rats of an inbred laboratory strain were used. The rats were kept housed in the same animal room and fed the same diet *ad libitum*; water was given freely. Treated female rats received 3 mg of testosterone propionate injected into the gluteal muscle every other day for a total of five injections (10-day course) in one group (6 animals), 12 injections (24-day course) in the other (6 animals). Control female rats received no injections. 48 h following the last dose of testosterone, the animals were killed by decapitation. The kidneys were removed and homogenized in 4 volumes of tris-(hydroxymethylaminomethane)-phosphate buffer 0.005 $M$, pH 7.25 in a Waring Blendor at 0–3°C, for 2 min. The homogenate was centrifuged at 0°C at 20,000 × $g$ for 30 min. The supernatant was decanted and dialysed for 18 h against

[1] Supported by NIH Grant and by the CNR.

[2] R. LEVI-MONTALCINI and P. U. ANGELETTI, International Symposium on Salivary Gland, Seattle (1962).

[3] H. E. BOND, Nature *196*, 242 (1962).

the original tris-phosphate buffer; column chromatography on DEAE-cellulose (No. 72 Selectacel Type 40-Lot D-1304) was carried out as previously described[2]. Three chromatographic fractionations were run for each experimental and control group. Protein was determined on each fraction by the method of LOWRY et al.[4] using crystalline bovine albumin as standard. In some experiments the optical density of each fraction was determined at 280 and 260 mμ in the Beckman DU Spectrophotometer.

In addition the activity of four enzymes was determined for each fraction. The enzymes were β-glucuronidase[5], acid phosphatase[6], alkaline phosphatase[6], and glucose-6-phosphate dehydrogenase (G-6-PDH)[7].

*Results and Comments.* Typical chromatograms of the soluble proteins in male, female and testosterone-treated female rat kidneys are shown in Figure 1. The major protein peak after the start of the salt gradient is arbitrarily numbered 10. Each peak or inflection point coming before peak 10 is then numbered, counting backwards. In addition there is a large peak following peak 10, with one or two smaller peaks intervening; the large peak is numbered 11. It was found that by carefully maintaining experimental conditions constant, a high degree of reproducibility for the location of at least four protein peaks (numbers 4, 8, 10, and 11) and the peaks of activity for the four enzymes studied could be obtained; variability was usually ±10 of eluate (±1 fraction). In male and testosterone-treated females there was a sharp separation of protein peaks 1, 2, and 4, whereas in normal females a plateau of protein concentration was reached after the gradient salt elution was begun. It was felt that chromatographic fine-structure of this degree did not represent a significant sex difference. The total protein in each successive 100 ml of eluate after the start of the gradient salt system is expressed as a percentage of all protein eluted with NaCl (Table). No quantitative difference in protein distribution between the male and the female chromatograms is noted.

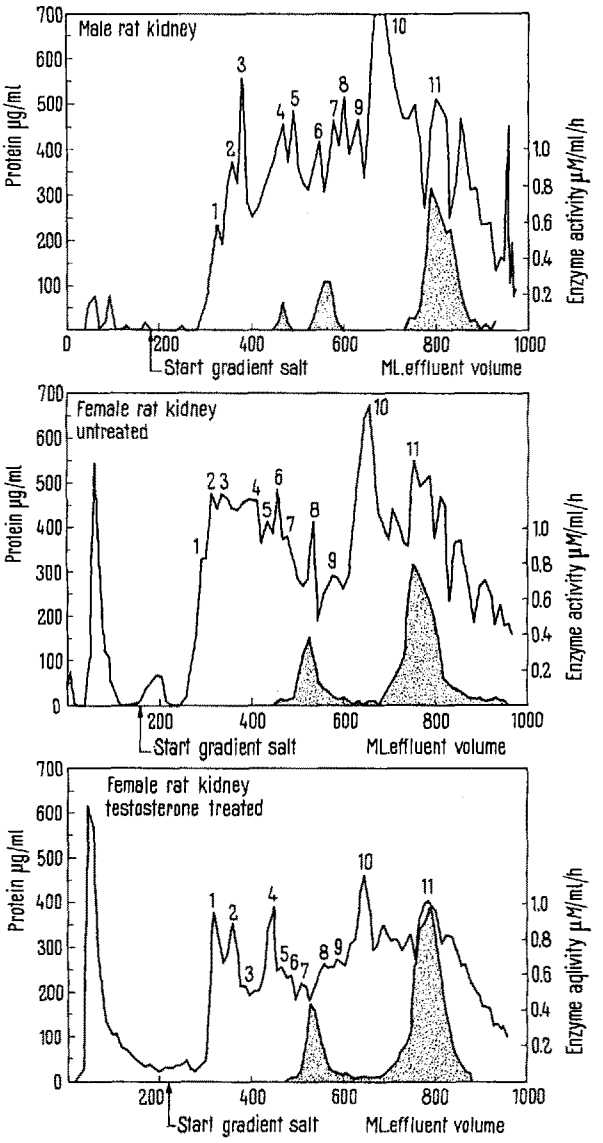


Fig. 1. Chromatograms of rat kidney extracts on DEAE-cellulose. Solid lines: proteins. Hatched area: Glucose-6-phosphate dehydrogenase (G-6-PDH) activity in μ moles/ml/h.

Four enzymes were localized in each chromatogram (Figure 1 and Figure 2). Two forms of G-6-PDH were distinguished; the first enzyme peak was found between protein peak 6 and 7, or between peak 7 and 8. The second enzyme form had highest activity in the fractions corresponding to protein peak 11. Both peaks characteristically were sharp. β-Glucuronidase was found in one form, always corresponding to protein peak 10, but spreading broadly over the centre of the chromatogram. Alkaline phosphatase was eluted in two broad peaks, the first corresponding to protein peak 3. The second was eluted in those fractions containing the last of the G-6-PDH activity; the two curves were sufficiently out of phase to obtain good separation. Acid phosphatase was found in three peaks in the male, the first corresponding to protein peak 1; the second to peak 6 and the third to the space between peaks 8 and 10. In both treated and untreated females the major acid phosphatase activity was located

Amount of protein in each successive 100 ml of eluate after start of salt gradient, expressed as a percentage of the total protein eluted with NaCl. Protein recovery was about 85%

| Pool number | % protein male | % protein female | % protein treated female |
|---|---|---|---|
| 1 | 0.56 | 4.07 | 2.41 |
| 2 | 11.85 | 17.72 | 14.97 |
| 3 | 14.75 | 14.98 | 14.07 |
| 4 | 15.34 | 10.81 | 13.41 |
| 5 | 21.75 | 18.45 | 19.09 |
| 6 | 18.17 | 17.23 | 18.86 |
| 7 | 14.24 | 12.59 | 13.37 |
| 8 | 3.35 | 4.15 | 3.80 |

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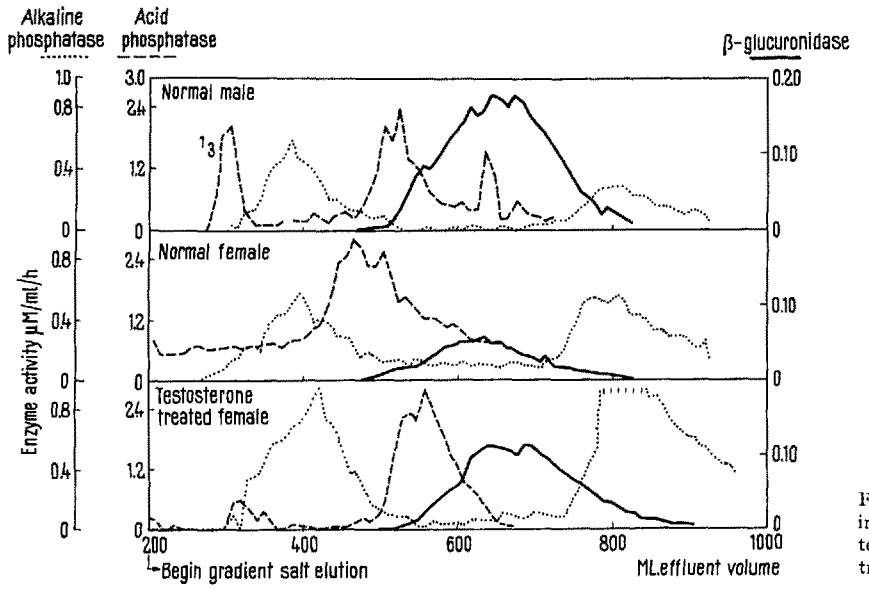


Fig. 2. Distribution of the enzyme activities in the chromatograms of male, female, and testosterone-treated female rat kidney extracts.

in a broad notched peak near the centre of the chromatogram, the first notch corresponding to the peak 6–7 space and the second to the peak 8–10 space. In the treated female a small amount of enzyme was eluted with protein peak 1; the activity was only 10% that of the male acid phosphatase in the same location.

The results presented above indicate that the sex-dimorphism of rat kidneys is not reflected in major, qualitative differences in their protein pattern.

Of the enzymes tested in our experiments, some were separated in multiple peaks; for each enzyme, however, the distribution of the multiple chromatographic forms was essentially the same in male and female pattern, with the exception of acid-phosphatase. The first peak of acid phosphatase activity was in fact missing in the female chromatograms and appeared upon testosterone treatment. It is conceivable that this particular form of acid-phosphatase in the kidney is under hormonal control. According to previous findings[8,9], it was found that the androgens influence the total activity of β-glucuronidase and of alkaline-phosphatase.

*Riassunto.* Sono state frazionate le proteine solubili estratte dal rene di ratto, mediante cromatografia su colonna di DEAE-cellulosa. Quattro enzimi sono stati localizzati nelle varie frazioni. Sono stati paragonati i profili cromatografici del rene del maschio, della femmina e della femmina sottoposta a trattamento con androgeni.

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## Alteration of Enzyme Activities in Detached Leaves and their Counteraction by Kinetin

Detached leaves are characterized by an intense protein breakdown and a respiratory drift[1]. The nature of respiratory drift, especially the mechanism of the increase in respiratory rate associated with the yellowing of tissues, is not fully understood. Kinetin, a compound capable of maintaining the normal protein level in detached leaves[2], appears to be a useful tool for the elucidation of the problem whether or not the respiratory changes taking place in detached leaves are causally connected with protein breakdown.

To throw some light on the possible mechanism of respiratory drifts in detached leaves, the level of a number of oxidative enzymes was measured according to conventional methods[3] in cell-free homogenates from detached and intact barley leaves. It has been shown that upon detachment the activity of a number of enzymes including glucose-6-P dehydrogenase, 6-P-gluconic dehydrogenase, isocitric dehydrogenase, malic dehydrogenase, dehydroascorbic acid reductase, peroxidase, inorganic pyrophosphatase markedly increases over the control. The most characteristic changes were experienced with the pentose phosphate shunt dehydrogenase. The activation of G-6-P dehydrogenase as a function of time after detachment is shown in Figure 1. It is also indicated that the increase in enzyme activity parallels the respiratory increase suggesting a possibility of causal relationship. Simultaneously

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