The glands removed from patients with breast cancer without previous ACTH stimulation have been considered as functionally normal on hand of clinical and laboratory data; histologically, they showed the three classical zones of the cortex, with a clear cut separation of the zona fasciculata into an outer clear-celled and an inner dark-celled layer. Histochemically, a different pattern of distribution has been observed according to the type of enzyme studied: a strong steroid-3β-ol dehydrogenase activity was evident in the clear cells of the fascicular zone and a weak one in some cell groups of the glomerulosa (Figure 1), whereas non-specific lipase and alkaline phosphatase were prominent in the dark cells of the zona reticularis.

A short-term intensive treatment with exogenous ACTH has increased in these glands the width of the enzyme activities, together with a lipid depletion of the clear cells. The steroid-3β-ol dehydrogenase activity was found to spread to the whole of the zona fasciculata (Figure 2), whereas the non-specific lipase and alkaline phosphatase activities extended outwards up to the outer zona fasciculata.

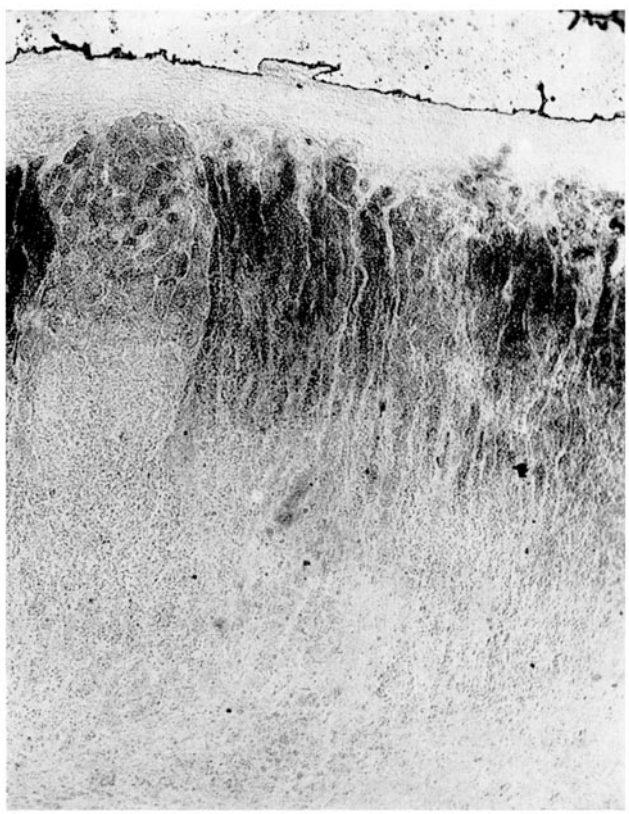

Fig. 3. Steroid-3β-ol dehydrogenase activity in adrenal cortex of a patient with Cushing's syndrome. ×60.

The findings in cases of Cushing's syndrome have been different according to their morphological back-ground. In the single case resulting from cortical tumour (adenoma), both clear and dark tumour cells showed a large quantity of all the enzymes studied; conversely, the tumour-free cortex was very poor or practically free of enzymes.

On the other hand, in cases resulting from hyperplasia of the adrenal cortex, no evident change in enzyme distribution has been observed; generally, the steroid-3β-ol dehydrogenase, as well as non-specific lipase and alkaline phosphatase, were within normal limits (Figure 3).

From the results outlined, the following conclusions may be drawn:

(1) In functionally normal glands, the presence of steroid-3β-ol dehydrogenase in the clear cells of the fascicular zone, as well as in cell groups of the glomerularis, supports the view that these elements are directly related to steroid-hormone synthesis or, at least, to an intermediate step of this process. Moreover, the presence in the reticular zone of some enzyme activities, such as non-specific lipase and alkaline phosphatase, poses the problem of the function of this zone, as it may be excluded that it is only a *zona consumptiva*.

(2) Exogenous ACTH leads to an increase of all the enzyme activities, which should be interpreted as a sign of functional stimulation.

(3) In Cushing's syndrome due to cortical adenoma, as expected, the tumour cells appear to be very active, whereas the tumour-free cortex is functionally at rest.

(4) Conversely, in Cushing's syndrome due to adrenal hyperplasia, the cortical enzymes show a pattern which is very near to normal or quite normal. This suggests that in this variety of the syndrome, there exists an interaction of some other still unknown factor or mechanism, apart from ACTH stimulation.

Zusammenfassung. Bericht über histochemische Untersuchungen an menschlichen Nebennieren, die chirurgisch bei Fällen von inoperablem Mammacarcinom (mit oder ohne vorhergehende Stimulation mit exogenen ACTH) sowie bei Fällen von Cushingscher Krankheit entfernt worden waren.

Anhand der Verteilung der verschiedenen Enzyme und insbesondere des Steroid-3β-ol Dehydrogenase wird auf Fragen der Beziehungen der Morphologie und Funktion der normalen Drüse eingegangen und die Cushingsche Krankheit interpretiert.

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Modifications of Myosin Caused by Adenosinetriphosphate, Adenosinediphosphate, and Pyrophosphate

In recent years, adenosinetriphosphate and pyrophosphate have often been used for the extraction of muscle proteins[1-3]. It was thought that they would help selectively extracting myosin from the myofibrils, but it was soon realised that, in addition to myosin, other protein material – extra-protein[4], X-protein[5], fraction T[6] – passes into solution and does not precipitate when the ionic strength is reduced to 0.04.

[1] W. R. AMBERSON et al., Biol. Bull. *99*, 314 (1950).
[2] W. HASSELBACH and G. SCHNEIDER, Biochem. Z. *321*, 462 (1951).
[3] J. HANSON and H. E. HUXLEY, Nature *172*, 530 (1953); Symp. Soc. exp. Biol. *9*, 228 (1955).
[4] A. G. SZENT-GYÖRGYI et al., Biochim. biophys. Acta *16*, 339 (1955).
[5] J. HANSON and H. E. HUXLEY, Biochim. biophys. Acta *23*, 250 (1957).
[6] I. I. IVANOV et al., Acta phys. Acc. Sci. Hung. *16*, 7 (1959).

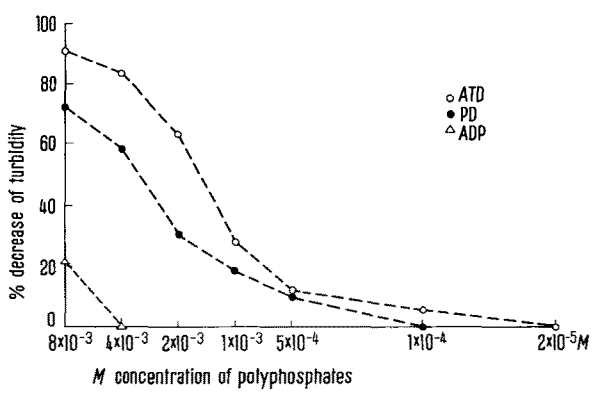
This fraction varies considerably, in quantity and composition, in relation to the experimental conditions. After extraction in the presence of adenosinetriphosphate or pyrophosphate, high figures were reported ranging from 11%[5] up to about 20%[4,6] of the total myofibrillar protein. This is difficult to reconcile with the results of the analysis of whole muscle[2], and also with the results obtained by other studies of isolated myofibrils[7-8].

The possibility that the extra-protein is in part some modified form of myosin was not excluded by PERRY and ZYDOWO[9] and by HANSON and HUXLEY[5]. Our experiments have been carried out with the aim of studying this possibility more closely.

Methods. Myosin was prepared by a method described by PERRY[10]; in most cases it was further purified by the ammonium sulphate fractionation procedure of DUBUISSON[11], as adopted by SZENT-GYÖRGYI[12]. Myofibrils were prepared from fresh muscle of the rabbit by PERRY's method[13]. Nitrogen was estimated by a conventional micro-Kjeldahl procedure. Adenosinetriphosphatase (ATPase) measurements were carried out at 25°C in a low ionic strength medium with 5 m*M* $CaCl_2$ and 2.5 m*M* ATP. Viscometry measurements were carried out at 0°C with the Ostwald viscometer. Total phosphorus was determined by the method of FISKE and SUBBAROW[14] after digestion with perchloric acid. Adenosinetriphosphate (ATP) and adenosinediphosphate (ADP) were supplied by Sigma Chemical Co. Pyrophosphate was supplied by Merck AG.

Results. We repeated the observations of BORBIRO[15] and of SPICER and WEISE[16], that ATP appreciably changes the solubility of myosin, so that it may remain in solution almost completely at low ionic strength.

ADP causes a similar change, but the concentration required is much higher. Pyrophosphate has the same effect: the level required is somewhat intermediate between those of ATP and ADP (Figure).



Effect of ATP, ADP, and pyrophosphate on the turbidity of a myosin suspension in 0.011 M KCl, 0.018 M borate buffer, pH 7. (Turbidity was measured at 600 mμ.)

Myosin in solution after treatment with different amounts of Na pyrophosphate and 20 h dialysis against 0.034 *M* KCl + 0.0025 *M* phosphate buffer (pH 6.9)

Initial concentration of pyrophosphate	% of total protein
0.01 *M*	9.7
0.02 *M*	37.2
0.04 *M*	55.1
0.08 *M*	56.3
0.16 *M*	70.4

We have confirmed the results of SPICER and WEISE[16] that Mg^{++} inhibits the effect of ATP on myosin, and we have observed that also the effect of pyrophosphate is inhibited by Mg^{++}.

ATP, ADP, and pyrophosphate, all appear to be bound to myosin very tightly, so that they can be only removed with difficulty on dialysis. If the initial concentration of added polyphosphate was high (0.01 *M* or more), dialysis over one night, against about 100 vol of a low ionic strength KCl solution, would not usually remove the polyphosphate completely so that an appreciable amount of myosin was still soluble at low ionic strength (Table).

Myosin dialysed over one night after treatment with 0.1–0.16 *M* pyrophosphate, showed a much reduced ATPase activity though the concentration of pyrophosphate left in the dialysis bag was very little, less than 1×10^{-4} *M*, certainly much below the amount which affects the enzymic activity of myosin when added to the incubation medium in the ATPase experiments. The viscosity of the solution was high at low ionic strength ($Z\eta \simeq 0.6$), but fell appreciably if the ionic strength was raised by addition of KCl or NaCl, as if there were a large amount of tropomyosin.

Only after a two days dialysis against a large amount of salt solution could the bound polyphosphate be removed so that myosin showed again its typical properties. (This result can be obtained more easily if Mg^{++} was added to myosin together with the polyphosphate.)

Experiments carried out with isolated myofibrils treated with concentrated pyrophosphate (0.1–0.16 *M*), have shown that as much as 75% of the protein extracted does not precipitate after over-night dialysis against a low ionic strength solution, but if dialysis is carried out for two days against 200 volumes of salt solution, the amount of materiel soluble at low ionic strength does not generally exceed 12% of the total protein extracted, and is always less than 10% of the protein content of the myofibrils.

It appears that using polyphosphates for extraction of myofibrils may lead to overestimates of the extra-protein fraction if the polyphosphates are not completely removed. An obvious corollary is that the figures of myosin extracted from the myofibrils are likely to be higher than they are often assumed to be[17].

Riassunto. In presenza di ATP o ADP o pirofosfato, in concentrazioni da 10^{-2} a 10^{-4} *M*, la solubilità della miosina aumenta notevolmente a bassa forza ionica. Il legame che si forma fra la miosina e i polifosfati è tale che solo una dialisi prolungata può allontanarli interamente.

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[7] S. V. PERRY and A. CORSI, Biochem. J. *68*, 5 (1958).
[8] A. CORSI, Biochim. biophys. Acta *25*, 640 (1957).
[9] S. V. PERRY and M. ZYDOWO, Biochem. J. *71*, 220 (1959).
[10] S. V. PERRY, in *Methods in Enzymology* (Ed. by COLOWICK and KAPLAN, Academic Press, New York 1955), vol. 2, p. 582.
[11] M. DUBUISSON, Exper. *2*, 10 (1946).
[12] A. SZENT-GYÖRGYI, *Chemistry of Muscular Contraction* (Academic Press, New York 1951).
[13] S. V. PERRY and T. C. GREY, Biochem. J. *64*, 184 (1956).
[14] C. H. FISKE and Y. SUBBAROW, J. biol. Chem. *66*, 375 (1925).
[15] M. BORBIRO, quoted by A. SZENT-GYÖRGYI, *Chemistry of Muscular Contraction* (Academic Press, New York 1951).
[16] S. S. SPICER and W. K. WEISE, Arch. Biochem. Biophys. *42*, 175 (1953).
[17] This research was aided by a grant from Muscular Dystrophy Associations of America, Inc.