Hiervon wurden an 0,1 ml die Absorptionsspektra in konzentrierter Schwefelsäure bestimmt. Die Maxima und Minima des Standards sowie der unbekannten Substanz lagen gleichförmig (Max. 240, 280, 390, 470, Min. 250, 340, 410 mμ, siehe [2]).

Die verbliebene Extraktmenge wurde mittels Dioxan[4] solvolysiert und im System Bush C rechromatographiert (Laufzeit 2,5 h bei 37°C. Rf-Werte für freies Cortisol und für die unbekannte Substanz 0,45). Nach Elution mit 80%igem Methanol erfolgte eine Chromsäureoxydation und eine Rechromatographie des Chloroformextraktes im System Formamid/Hexan-Benzol, 1:1 (Laufzeit ca. 3 h bei Zimmertemperatur. Lokalisation der Flecken mittels UV-Kontaktphoto und Zimmermannreaktion). Der Rf-Wert der unbekannten Substanz entsprach dem des Adrenosteronstandards (0,4). Nach erneuter Elution mit 80%igem Methanol wurden die Absorptionspektra in konzentrierter Schwefelsäure gemessen, die mit dem des Adrenosteronstandards übereinstimmten (Max. 285, Min. 245 mμ).

Diskussion. Mehrere Autoren[5-7] haben den Nachweis erbracht, dass die menschliche Nebennierenrinde Cortisol mit Sulfat konjugieren kann. Im Nebennierenvenenblut sowie in der V. hepatica des Menschen konnte OERTEL[6] jedoch nur sehr geringe Konzentrationen dieses Steroidkonjugates finden, die eine exakte Identifizierung nicht erlaubten. Wie die oben dargestellten Ergebnisse zeigen, liess sich Cortisol-21-sulfat im Urin von Cushingpatienten sowohl vor wie nach einer totalen Adrenalektomie mit oraler Cortisolsubstitution identifizieren. Cortisol-21-sulfat kann daher beim Menschen mit Sicherheit auch extraadrenal gebildet werden. Ob beim Menschen, ähnlich wie bei der Ratte[8], neben der Leber auch die Nieren zur Konjugierung von Cortisol mit Sulfat imstande sind, muss noch offen bleiben.

Summary. Cortisol-21-sulphate has been identified in the urine of three patients with Cushing's syndrome before and after total adrenalectomy (substitution with 200 mg F orally). The identification was based upon the following data: Identical behaviour of the standard cortisol-21-sulphate and the unknowns in two different paper chromatographic systems; positive methylene blue reaction and UV-contact-photogram; identical absorption spectra in concentrated sulphuric acid; correspondence with free cortisol of the steroid moiety liberated by dioxan solvolysis and formation of adrenosterone following treatment with chromic acid. The data support evidence that cortisol-21-sulphate may originate not only from the human adrenal gland but also from extra-adrenal sources, presumably the liver[9].

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Myoinositol Excretion in the Newborn Infant[1]

The 'inhibition assay'[2] principle has been used to develop a number of agar-diffusion microbial tests. Biological specimens of interest were compared by placing impregnated paper discs on the surface of an agar culture medium containing a microorganism whose growth is prevented by an appropriate inhibitor. These tests have been applied to human urine for the detection of metabolites associated with various diseases or with human development. In the process of testing the urine of new-born infants in this manner, employing *Saccharomyces cerevisiae* inhibited by 5-fluorouracil ($7 \cdot 10^{-6} M$), an activity was observed similar to that produced by cytosine. Infant urine prevented the inhibition of the yeast by 5-fluorouracil, whereas it failed to prevent such inhibition of *Bacillus subtilis*. In contrast uracil prevented 5-fluorouracil inhibition of both organisms. Subsequent paper chromatography of a sample of pooled new-born infants' urine clearly demonstrated that the unknown substance was neither uracil nor cytosine. The inhibition assay of urine specimens from fifty individual new-born infants showed that this activity was uniformly present. Similar investigations of individual healthy adults failed to reveal appreciable amounts. However, the activity was found associated with generalized aminoaciduria due to different causes in a number of children. The above data led us to undertake the isolation and identification of the compound.

Preliminary purification by paper chromatography employing three different solvent systems, followed by ion-exchange chromatography led to a partially purified sample which, unlike purines and pyrimidines, absorbed only slightly in the ultraviolet. A comparison with the tables of Rf values compiled by FINK, CLINE, and FINK[3] indicated that the compound had properties similar to myoinositol. Additional chromatographic comparison of the unknown with an authentic sample confirmed the above observation. In a myoinositol-deficient culture medium, the unknown substance and myoinositol each

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produced growth of *S. cerevisiae*, either in the presence or in the absence of 5-fluorouracil. The ability of myoinositol to promote growth of *S. cerevisiae* is well known[4].

When the concentration of 5-fluorouracil was increased in the culture medium from the usual level of $7 \cdot 10^{-6}$ to $10^{-4} M$, myoinositol[5] no longer was effective in restoring growth. Cytosine[5] and uracil[5] were both active under these conditions, with the former significantly more active than the latter. These results in yeast may be related to the role of cytidine diphosphate in the biosynthesis of myoinositol phosphatides in the mammal[6].

To our knowledge, this is the first report indicating a higher excretion of myoinositol by the new-born infant than by adults. Although work has been done on adult excretion of myoinositol[7–12], the only report dealing with children is the early observation of KULZ[13]. He concludes that the urine of adults and children with a wide variety of diseases may contain myoinositol. It has been shown that the myoinositol content of fetal blood and fetal fluids is consistently higher than in the mother[14].

Zusammenfassung. Ein aktives Agens im Säuglingsurin, das die Hemmung von *Saccharomyces cerevisiae* durch 5-Fluorouracil verhindert, wurde als Myoinositol identifiziert und generell bei allen Neugeborenen, ebenfalls bei einer Anzahl von Kindern mit allgemeiner Aminoacidurie, gefunden. Gesunde Erwachsene zeigten keine nennenswerten Mengen.

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Studies on the Chemical Composition of Streptococcal Co-opsonin

It has been reported recently that absorption of human serum with bentonite results in removal of a non-specific 'co-opsonin', which is necessary for optimal phagocytosis of virulent group A streptococci by polymorphonuclear leucocytes in the presence of type-specific antibodies[1,2]. Amounts of bentonite which almost completely inactivated the serum opsonic activity did not reduce significantly either complement, properdin, or any of the major components of the complement system. This paper describes attempts to characterize the chemical nature of this co-opsonin. It will be shown that it is distinct from lysozyme, and that it may reside in the serum β-lipoprotein moiety.

Materials and methods. Removal of the co-opsonin: Fresh serum from the same donor (M.W.R.) collected in a fasting state, was used in all experiments. The procedure of co-opsonin absorption has been described in detail in a previous communication[2] and will be summarized only briefly. Serum was incubated at 37°C for 20 min with the desired amounts of bentonite. It was then separated by centrifugation (6800 × *g* for 30 min at 4°C in a Model-L Spinco ultracentrifuge with a No. 30 rotor head) and stored at −70°C until ready for use. Stock suspensions of bentonite were prepared by adding the dry powder (Bentonite, U.S.P., Fischer, Lot No. 790219) to distilled water in a concentration of 3 mg per ml. Particle dispersion was improved by mixing the suspension in a Waring blendor for 1 min. Bentonite in amounts desired for serum absorption was separated from stock suspension by centrifugation (2800 × *g* for 30 min).

The phagocytic test: The phagocytic test ('Bactericidal Test') was identical with that employed in previous studies[1,2]. Overnight Todd-Hewitt broth cultures of a strain of type 6 group A streptococcus (T-6/543) adjusted to the desired concentration, type-specific rabbit antiserum, fresh human serum (M.W.R.) as source of natural co-opsonins, and autologous polymorphonuclear leucocytes, twice washed in physiologic saline, were employed in all experiments. The degree of phagocytosis was expressed by the 'bactericidal index' (BI), which is determined by the relative inhibition of the growth of the streptococcus in the phagocytic test; i.e.

$$\text{Bactericidal index} = \frac{\text{No. of organisms in the inoculum}}{\text{No. of organisms surviving phagocytosis in the presence of type-specific antibody and test serum}} \times \text{Growth in absence of type-specific antibody}$$

As is apparent, the greater the degree of destruction of streptococci by phagocytosis, the greater the numerical value of the bactericidal index.

Complement titration: Complement titration was carried out to the 50% hemolytic endpoint according to the method of KABAT and MAYER[3].

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