produkte wurden erhalten. Der gesuchte Stoff befand sich zur Hauptsache in den Fraktionen 32–39, die aus Azeton–Äther kristallisierten; Spuren liessen sich noch in den Fraktionen 31 und 40 nachweisen. Die Ausbeute an Rohkristallen betrug 22 mg. Umkristallisieren lieferte 21,2 mg farbloses, einheitliches, aschefreies Material. Aus Azeton–Wasser wurden besonders gut ausgebildete Kristalle erhalten (vgl. Abb. 1). Die lufttrockenen Kristalle enthielten Kristallwasser, denn bei längerem Trocknen bei 0,01 Torr und 50° über P_2O_5 wurde 1 Mol H_2O abgegeben. Das Hydrat schmolz auf dem Kofler-Block je nach Kristall-grösse und Erhitzungsgeschwindigkeit im Bereich zwischen 104 und 112° innerhalb etwa 3°. Die Masse kristallisierte bei weiterem langsamem Erwärmen wieder vollständig und schmolz dann bei etwa 153–158° definitiv. (Die letzten Spuren verschwanden oft erst bei rund 165°). Die spezifische Drehung des Hydrats betrug $[\alpha]_D^{23} = +145° \pm 2°$ (c = 0,9896 in Azeton), das UV.-Absorptionsspektrum (vgl. Abb. 2) zeigte die für α,β-ungesättigte Ketone typische Bande.

Die beschriebene Verbindung war im Test nach SIMPSON und TAIT[2] rund 100mal und in dem von DESAULLES und SCHULER modifizierten Natriumretentionstest nach KAGAWA *et al.*[3] etwa 50mal stärker wirksam als Cortexon. Im Erhaltungstest am nebennierenlosen Hund erwies sie sich als mindestens 30mal wirksamer als Cortexon-*azetat*[4]. Die Ergebnisse der beiden zuletzt erwähnten Versuchsanordnungen sprechen dafür, dass sich die Wirkungen der Verbindung auch in qualitativer Hinsicht von denjenigen des Cortexon unterscheiden. Wir glauben daher, dass ein neues wichtiges Hormon der Nebennierenrinde vorliegt. Über die Versuche zur Konstitutionsermittlung und Synthese wird später berichtet, ebenso über eingehendere biologische Versuche. Wir behalten uns vor, für die Verbindung dann einen neuen Trivialnamen vorzuschlagen.

Wir danken Herrn Prof. E. C. DODDS aufrichtig für sein Interesse an dieser Arbeit und die Förderung des Kontaktes zwischen den Arbeitsgruppen in London und in Basel. Der N. V. Organon Oss, Holland, danken wir auch hier bestens für die Überlassung von Nebennierenextrakten.

S. A. SIMPSON, J. F. TAIT,
A. WETTSTEIN, R. NEHER,
J. v. EUW und T. REICHSTEIN.

The Middlesex Hospital Medical School, London W. 1; Forschungslaboratorien der CIBA Aktiengesellschaft, Basel, Pharmazeutische Abteilung, und Organisch-Chemische Anstalt der Universität Basel, den 23. Juli 1953.

[1] I. E. BUSH, Biochem. J. *50*, 370 (1952). – Diese Säule mussten wir sehr gründlich vorreinigen und die Lösungsmittel jeden Tag frisch destillieren, trotzdem verblieb ein merklicher Blindwert (Autoxydation des Toluols?).

[2] S. A. SIMPSON und J. F. TAIT, Endocrinology *50*, 150 (1952).

[3] C. M. KAGAWA, E. G. SHIPLEY und R. K. MEYER, Proc. Soc. Exp. Biol. Med. *80*, 281 (1952). Wir danken den Herren Dr. P. DESAULLES und Prof. W. SCHULER auch hier bestens für die Ausführung dieser Bestimmung, bei der zusätzlich die Wasserretention sowie die Veränderung der Kaliumausscheidung gemessen wurde[5].

[4] G. A. HARROP, J. J. PFIFFNER, A. WEINSTEIN und W. W. SWINGLE, Proc. Soc. Exp. Biol. Med. *29*, 449 (1932). – J. J. PFIFFNER, W. W. SWINGLE und H. M. VARS, J. Biol. Chem. *104*, 701 (1934). – R. MEIER, H. GYSEL und R. MÜLLER, Schweiz. Med. Wschr. *74*, 93 (1944). – F. GROSS und R. MEIER, Schweiz. Med. Wschr. *81*, 1013 (1951). – Herrn Dr. F. GROSS sei auch hier bestens für diese Bestimmung gedankt[5].

[5] Die genannten Herren werden über ihre Versuche an anderer Stelle ausführlicher berichten.

Summary

A new crystalline compound has been isolated from beef adrenal extracts. In three different assay methods using epinephrectomised rats or dogs it was found to be from 30 to 100 times as potent as cortexone (11-deoxycorticosterone) or its acetate.

The Gram-Staining Behavior of Spermatozoa

One of the earliest observations on the gram-staining of spermatozoa was made by ERNST[1] who noticed that the heads of spermatozoa stain grampositively. Five decades later, in 1944, HOTCHKISS[2] recommends "that the smear be flamed" prior to the use of gram-stain. Then he adds, "some of the spermatozoa take the gram-positive and some the gram-negative reaction". Finally, he says "the interpretation of this reaction is unknown to the author". This inconclusiveness led us to re-examine the gram-staining behavior of spermatozoa.

Materials and Methods

Smears.—Samples of human semen 15 min old and thus liquefied[3] were used, and the smears made according to the method of HOTCHKISS[2].

Fixation.—Either air drying, flame fixation or an immersion for 2 min in a mixture of formalin[4] and ethyl alcohol (95%) 1:9 was used. After fixation by any of these methods the slides were kept in ethyl alcohol (80%) prior to staining. (The immersion in 80% ethyl alcohol should not exceed 1 or 2 h).

Gram-Stain.—This was made up according to ROULET[5]. The "mordant" was prepared by dissolving 1 g iodine[6] in 100 ml of ethyl alcohol (80%). As decolorizer ethyl alcohol (95%) was used.

Gram-Staining Procedure.—The slides kept in ethyl alcohol (80%) were transferred for a few seconds to a 70% ethyl alcohol solution, then to one of 50% concentration, and finally to tap water. They were then immersed in the gentian violet solution for 2 min. After rinsing three times in tap water (fresh changes each time) the slides were immersed for one second in ethyl alcohol (50%) and then in the "mordant" for 30 s. After rinsing by 1 s immersions successively in 3 COPLIN jars containing ethyl alcohol (80%), the slides were kept 2 min in ethyl alcohol (95%) in order to be decolorized and then rinsed in absolute alcohol for a few seconds and dried before proceeding with microscopic examination.

Other Treatments Prior to Gram-Staining.—Fixed slides were rinsed successively in 80%, 70%, 50% alcohol, then in tap water for a few seconds and finally, kept in 0·5% aqueous picric acid solution for 20 h at room temperature. Some of these slides were treated with GRAM's gentian violet solution for 23 h. Control slides were kept in alcohol (80%) during the time of treatment, i.e., after fixation and prior to the application of gram-stain.

[1] P. ERNST, Arch. mikroskop. Anat. *47*, 669 (1896).

[2] R. S. HOTCHKISS, *Fertility in Man* (Lippincott, Philadelphia, 1944).

[3] J. MACLEOD, Ann. N. Y. Acad. Sci. *54*, 796 (1952).

[4] From Ingram & Bell Ltd: pH = 4·5; strength = 39 to 40%.

[5] F. ROULET, *Methoden der pathologischen Histologie* (Springer, Wien 1948), p. 480. – With gentian violet of the Ciba Company Ltd.

[6] There is no reason to add potassium iodide since iodine is quite soluble in ethyl alcohol (80%) without the presence of KI. An aqueous solution of iodine is impractical for several reasons: KI must be present to render the iodine soluble, the watery solution causes a dissociation of tissues and the penetration of iodine into the tissues is slower.

Results

The gram-staining response of spermatozoa depends on the fixation method or other treatments used prior to the staining procedure. Thus, air drying of the slides from 6 min to 1 h with or without additional flame fixation leads to gram-negativeness. On the other hand, fixation for 2 min in formaldehydealcohol prior to staining gives a gram-positive response. Slides thus fixed and then kept in 0·5% picric acid solution for 20 h are converted to gram-negativeness again when exposed to the gram-staining procedure. Such slides, however, if kept in GRAM's gentian violet solution for 23 h prior to the regular gram-staining are reversed to gram-positiveness again.

The gram-positive reaction of spermatozoa seems to be concentrated at the base of the head and neck.

Discussion

The results obtained show that oxidizing agents or acids (air drying, picric acid (0·5%) if applied prior to staining lead to a gram-negative response of the spermatozoa while reducing agents have an opposite influence.

In previous articles[1] it was established that intact wool which shows a gram-negative behavior can be reversed to a gram-positive one by reducing or alkaline agents and then reconverted again by means of oxidizing agents or acids to its original gram-negativeness. The behavior of such fibrous proteins showing an α-keratin structure was described by the authors to be analogous to that of the cytoplasmic *membrane* of gram-negative bacteria and gram-positive bacteria respectively.

It can be shown that the gram-staining response is a characteristic phenomenon of keratin structures in histological specimens[2]. The gram-positiveness observed seems to be associated with the presence of SH groups either in the pre-formative keratin state in the hair follicle or in the more matured (degraded) keratin parts of hair. Gram-negativeness, on the other hand, is apparently associated with the presence of –S–S– groups from the cystine in intact keratins.

However, the reduction-oxidation-scheme mentioned above[3] is not applicable to the keratin in epidermal tissue sections, the gram-positiveness of which cannot be reversed[4]. That scheme is valid only for keratins present in alkali-degraded wools, matured hair and nails. Since according to BLOCK[5] the cystine content of the epidermal keratin is only 3·5 g (per 16 g of N) and that of hair and nails 16 and 13 respectively, we conclude tentatively that the reversibility of the gram-positiveness is characteristic of keratins with high cystine content and that keratins with a low cystine content fail to show this reversibility in behavior.

Electromicroscopic studies of SCHNALL[6] revealed recently a membrane sheath located at the lower part of the head and neck of the spermatozoa. DANIELLI[7] describes that area as being constituted of protein. Indeed the presence of basic nuclear proteins was confirmed throughout the whole of the head of ram spermatotoa[1] but the general protein reaction was found to be concentrated near the base of the head. Since our results also show mainly the same area to react like certain keratins after exposure to the gram-staining procedure, we assume the structure to be that of a keratin. The high sulphur content of spermatozoa[2] is further supporting evidence for our assumption since keratins with a reversible gram-staining response have as one of their characteristics a relatively high percentage of sulphur.

The gram-staining behavior of the membrane sheath of spermatozoa does not depend only upon treatment prior to the gram-staining procedure. Seminal fluid which has a pH of 7·4 to 7·6[3], gets more and more alkaline on standing since more and more CO_2 is lost from the sample. Therefore, it is to be expected that the older the sample, the more the alkalinity developed renders the keratin membrane of the head of the spermatozoa gram-positive. The results are also at variance if the spermatozoa are collected from the introitus vaginae, since this acidic milieu (up to pH 3·5) may render them gram-negative or, if the seminal sample is taken from the cervical part (pH 9·6) where the alkalinity might render the spermatozoa gram-positive.

It should be noted that fibrous keratin exposed to alkalinity for a sufficient length of time has a lowered tensile strength and exhibits a marked susceptibility to proteolytic enzymes[4]. The presence of such a degraded keratin in the lower part of the head and in the neck of the spermatozoa as the result of the influence of cervical alkalinity, may facilitate the loss of the tail from the head after fertilization of the ovum.

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Zusammenfassung

Der Einfluss der Vorbehandlung auf das Gramfärbeverhalten menschlicher Spermatozoen wurde untersucht. Die Membranhülle des Spermienkopfes sowie auch die Fibrillen des Halses weisen ein für bestimmte Keratine charakteristisches färberisches Verhalten auf. Vorbehandlung mit Reduktionsmitteln lässt eine Grampositive, Vorbehandlung mit Oxydationsmitteln eine Gram-negative Färbung resultieren. Einige aus diesem Verhalten sich ergebende Möglichkeiten werden diskutiert.

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