podophyllic acid (−165°) and DPP (+43°). The observation[2] that isoanthricin was insoluble in cold caustic alkali is not inconsistent with the behavior expected from a mixture containing some alkali-insoluble lactone.

Hernandion and Isohernandion.—Hernandion was isolated by Hata[3] from the seed oil of *Hernandia ovigera* L. (Fam. *Hernandiaceae*). Direct comparison[3] of hernandion and isohernandion with anthricin and isoanthricin, respectively, showed no mixed melting point depression. The reported physical constants (Table I) also agree with ours for DPT and DPP, respectively. The identity of hernandion and isohernandion with the latter compounds is thus clear.

There remains only the question of the failure of isoanthricin to depress the melting point of isohernandion if the former was composed largely of desoxypodophyllic acid. We have found[1] that slow heating of the hydroxy acid in the melting point tube brings about complete lactonization to DPP. It is therefore quite possible that under certain conditions such a mixed melting point would show no depression.

Cicutin.—This lactone was isolated by Marion[4] from the roots of *Cicuta maculata* L. (Fam. *Umbelliferae*) (water hemlock). Although no chemical evidence, apart from the empirical formula and the identification of the substituent groups, was available, a sample of cicutin kindly provided by Dr. Marion, found by us[1] to have m.p. 168.7–169.4°, gave an infrared spectrum essentially identical with that of DPP, and the opinion was expressed that cicutin might be largely DPP. The optical rotation, however, showed that if it was DPP it was not pure. The sample was therefore refluxed with methanolic sodium acetate to epimerize any possible residual DPT, and chromatographed on alumina, which removed some ultraviolet-fluorescent material. The product thus obtained (colorless electrified needles,[1] from methanol) had m.p. 171–172°, which was not depressed on admixture with DPP, and $[\alpha]^{20}D$ + 49° (*c* 0.24, pyridine), corresponding to the optical rotation of DPP.

The question of whether cicutin, which was extracted from the plant by means of methanolic sodium hydroxide, is an artifact or not cannot be resolved at this time. Either DPT or DPP originally present in the plant would be expected to yield DPP by the method of isolation used. Since *Cicuta maculata* L. and *Anthriscus sylvestris* Hoffm. are both *Umbelliferae*, and since lignans belonging to the "picro" series are extremely rare in nature, it is a reasonable assumption that cicutin exists originally in the plant in the form of its epimeric precursor, DPT.

In summary, the naturally-occurring lignans anthricin, hernandion and silicicolin have all been shown to be identical; it is proposed to discard all these names in favor of desoxypodophyllotoxin, a name which indicates the chemical and steric relationship to podophyllotoxin, the first lignan of this type discovered. Cicutin has been shown to be identical with desoxypicropodophyllin.

Acknowledgment.—The authors wish to express their sincere thanks to Dr. Léo Marion, National Research Council, Canada, for a sample of cicutin; to Dr. M. Kawanami, Osaka, Japan, for samples of anthricin and isoanthricin; to Mrs. Priscilla B. Maury for determining the optical rotations; and to Mrs. Iris J. Siewers for measuring the infrared spectra.

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(3) C. Hata, *J. Chem. Soc. Japan*, **63**, 1540 (1942). This paper was called to our attention by Mr. M. E. Cisney of Crown Zellerbach Corp. Through an error in abstracting [*C. A.*, **41**, 2917b (1947)], this compound (under an erroneous systematic name) was given the empirical formula $C_{21}H_{32}O_6$, and so was missed in our earlier literature survey. A correction abstract was carried by *C. A.*, **47**, 10872c (1953). This paper does not appear to have been abstracted by any of the other chemical abstract journals.

(4) L. Marion, *Can. J. Research*, **20B**, 157 (1942).

The Separation and Characterization of Serum Albumin from Extracts of a Lymphatic Organ[1]

BY E. L. HESS, MILDRED CAMPBELL AND AILENE HERRANEN

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In the course of the chemical fractionation of bovine palatine tonsils we have isolated in a relatively pure form one of the components present in the initial extract. Characterization of the substance reveals that many of its properties are identical with those of bovine serum albumin. The purpose of this communication is to report that serum albumin seems present in considerable quantity in the cytoplasmic extracts of a lymphatic organ.

Experimental

Details concerning procedures used in electrophoretic, sedimentation velocity, spectrophotometric and nitrogen analyses have been published previously.[2] Unless otherwise specified all electrophoretic patterns are photographs of descending limbs after 120 minutes under a potential gradient of 6.4 volts cm.$^{-1}$ in veronal buffer *p*H 8.6, μ 0.10. A modified Ouchterlony technique[3,4] was used in the serological comparison.[5] Details of the earlier steps in the fractionation procedure have been published.[2] The component labeled M2 in Fig. 2A, B, C, of the earlier publication has been considered in the present investigation. The starting material in this report has been the *p*H 4.2 supernatant (Spnt. C) in Fig. 1 of the above mentioned report.[2] This supernatant when lyophilized, yielded about 0.6–0.8 g. of solids for each 100 g. of fresh tonsil; this amounts to about 5% of the dry weight of the whole organ and to about 14% of the cytoplasmic extract. The electrophoretic pattern of the starting fraction (4.2S) is shown in Fig. 1A. The albumin peak amounts to approximately 30% of the pattern.

Five grams of lyophilized 4.2S solids was dissolved in one liter of distilled water (2°) and the *p*H adjusted to 6.9 with 0.05 *N* NaOH. To this solution was added 82 g. of solid ammonium sulfate and 1000 ml. of cold saturated ammonium sulfate (70 g. of solid ammonium sulfate in 1000 ml. of water at 5°). The *p*H of this solution was 6.0. The solution was stirred 20 minutes and the precipitate removed by centrifugation. This precipitate called 6P amounted

(1) This report represents work done under contract with the U. S. Atomic Energy Commission, Project No. 6 to Contract AT(11-1)-89 with Northwestern University.

(2) E. L. Hess, W. Ayala and A. Herranen, THIS JOURNAL, **74**, 5410 (1952).

(3) O. Ouchterlony, *Lancet*, **2**, 346 (1949).

(4) R. K. Jennings, *J. Immunol.*, in press.

(5) We are grateful to Dr. Jennings of this Institute who kindly performed the serological studies.

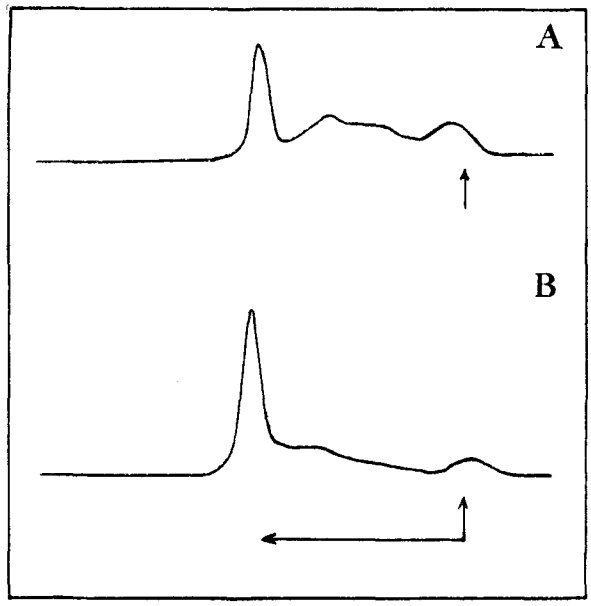

Fig. 1.—A, starting fraction 4.2S; B, fraction 4P.

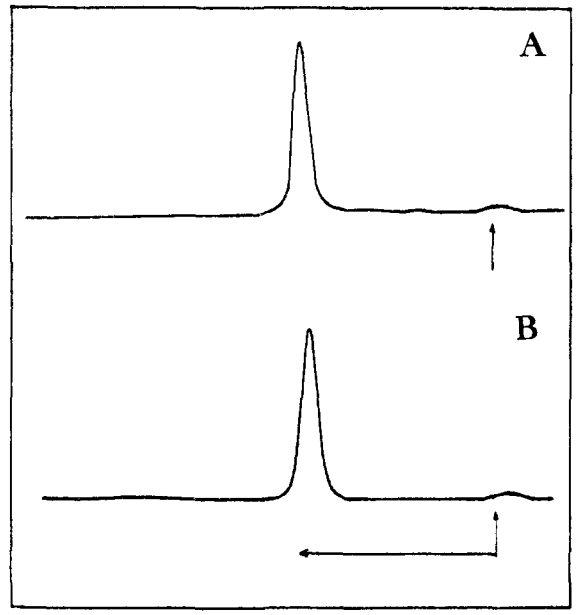

Fig. 2.—A, bovine tonsil albumin; B, bovine serum albumin.

to 0.98 g. The *p*H of the supernatant was lowered to 4.0 with 0.1 *N* sulfuric acid. The resulting precipitate, called 4P, obtained by centrifuging the *p*H 4 suspension, when dissolved in water, dialyzed and lyophilized amounted to 1.6 g. This precipitate gave the electrophoretic pattern seen in Fig. 1B. The albumin peak constituted about 70% of the area of this pattern. The supernatant was dialyzed and lyophilized yielding 1.2 g. of solids. The loss of material occurring in the above fractionation is ascribed chiefly to proteolysis due to the presence in 4.2S of a proteinase with an optimum activity at *p*H 3.[6]

It was found that hydrolysis of the albumin occurred when the 4P fraction was allowed to stand at acid *p*H values. In order to separate albumin from the proteinase, fraction 4P (lyophilized) was extracted with a solution of 0.7 saturated ammonium sulfate at 5° at *p*H 6. Four successive extractions were performed on 1 g. of 4P using 100 ml. of 0.7 saturated ammonium sulfate for each extraction. The contents of the first two extracts were precipitated by saturating the solution with solid ammonium sulfate. After centrifugation, the precipitate (4P70S), dissolved in water, dialyzed and lyophilized, amounted to 220 mg. The residue after four extractions was dissolved in water, dialyzed and lyophilized. This residue yielded 120 mg. of dried material and was found to be rich in proteolytic activity for which crystalline bovine serum albumin was found to be a suitable substrate.[6] The electrophoretic pattern given by 4P70S is shown in Fig. 2A. The properties of this material will be discussed below.

Comparison of the electrophoretic behavior of bovine tonsil albumin (BTA) and crystalline bovine serum albumin[7] (BSA) was made at *p*H 8.6 in 0.10 ionic strength (μ) veronal buffer as is shown in Fig. 2. Figure 3A is the pattern of BSA in μ = 0.10 acetate buffer at *p*H 4.70 whereas Fig. 3B is a mixture of equal amounts of BSA and BTA in the same buffer.

Rabbit antiserum prepared by immunizing rabbits against BSA contained antibody which reacted with both BSA and BTA, as indicated by the double diffusion precipitate pattern. The concentration of BSA and BTA used was identical in the two wells of the Ouchterlony plate. Precipitate patterns formed by a common antibody source reflect differences in concentration of antigen. No significant difference was noted in these experiments. We interpret this as evidence that the amount of reactive antigen present in BTA was not noticeably different from that in BSA.

In Table I are tabulated additional characterization data. The extinction coefficients reported are based on weight of sample dried at 105° *in vacuo*. Our uncorrected value of

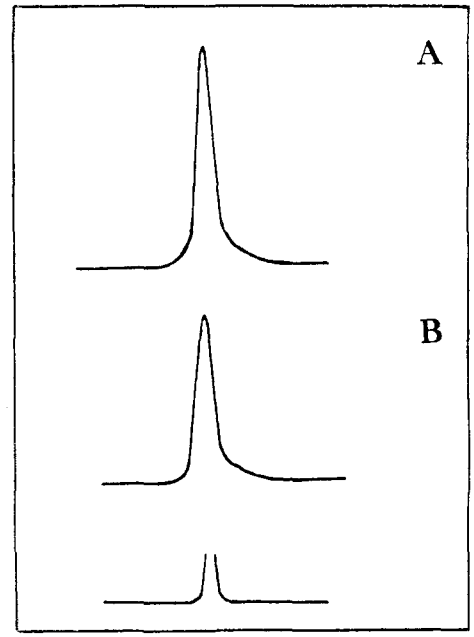

Fig. 3.—A, electrophoretic pattern of BSA after 120 minutes under a potential gradient of 4.0 volt cm.$^{-1}$ in *p*H 4.70, μ = 0.10 Na acetate; B, a mixture of equal parts BSA and BTA under the same conditions as in A.

$E^{1\%}_{280}$ 6.6 for BSA is identical with that given by Cohn, Hughes and Weare.[8] Since these authors did not indicate whether the value they reported for $E^{1\%}_{280}$ was corrected for moisture loss at 105° *in vacuo*, it is presumed that their value is uncorrected.

The sedimentation coefficients reported are based upon single sedimentation experiments and have not been extrapolated to zero protein concentration. Both experiments were performed the same day under experimental conditions as identical as possible. In each instance a 1% solution of the protein was prepared in 0.15 *M* NaCl at *p*H 4.7. The reported values are in agreement within the limit of error

(6) E. L. Hess, M. Campbell and A. Herranen, unpublished results.

(7) Obtained through the courtesy of Dr. Lawrence Lachat of Armour and Co., Chicago; BSA Control No. 51.

(8) E. J. Cohn, W. L. Hughes, Jr., and J. H. Weare, THIS JOURNAL, 69, 1753 (1947).

TABLE I

COMPARISON OF CHARACTERIZATION DATA ON BOVINE SERUM ALBUMIN AND ALBUMIN PREPARED FROM BOVINE PALATINE TONSIL EXTRACTS

	BSA	BTA
$E_{280}^{1\%}$ [a]	7.02	7.00
N_2, % [b]	16.0	16.0
s_{20w} [c]	4.5S	4.2S
$\mu \times 10^5$ [d]	−6.64	−6.50
pI_e [e]	4.70	4.70

[a] Slit setting 0.6. [b] This value is based on weight of sample dried at 105° *in vacuo*. [c] Sedimentation measurements were made in the laboratory of Prof. J. W. Williams at the University of Wisconsin. [d] *p*H 8.6 μ = 0.10 veronal. [e] *p*H 4.70, μ = 0.10 acetate.

of the method.[9,10] It is of interest that BTA gave a single symmetrical peak while in BSA there appeared a small shoulder of more rapidly sedimenting material.

The isoelectric point of 4.70 is in agreement with the studies of Baldwin, Laughton and Alberty[11] who report a value of 4.71 for BSA in the same buffer as we have used.

Discussion

The agreement between the characterization data for BSA and BTA leaves little doubt that the material prepared from bovine tonsils is physically, chemically and serologically identical with albumin prepared from serum.

Our studies do not provide information concerning the possibility that the albumin is an intracellular constituent of the lymphocyte. Lymphatic tissues are bathed in lymph, the composition of which is similar to serum.[12] Therefore, it is not surprising to find albumin the major component of serum, present in extracts of a lymphatic organ. It should be emphasized that the tonsils used for extraction are almost free of blood. For this reason the presence of albumin in the quantity noted in these extracts cannot be dismissed as a contamination resulting from blood. The method of procuring glands free of traumatized blood has been discussed.[2] A further check on this point is provided by the hemoglobin content of the initial extract. Only traces of hemoglobin are present in the original extract. Another factor pertinent to this discussion is the relatively constant amount of BTA found in the extract. We have examined this point repeatedly and find little variation in the BTA content in different fractionations. Also of interest in this connection is the work of Abrams and Cohen.[13] These workers using different extraction procedures and both human tonsils and calf thymus obtain electrophoretic patterns of the initial extracts almost identical with those we have obtained from bovine tonsils. Abrams and Cohen have suggested that component 5 in their patterns was probably serum albumin, which our work confirms. Approximately 5% of the cytoplasmic extract of the tonsil consists of serum albumin irrespective of whether this constituent is present as an intracellular or as an extracellular constituent.

(9) S. Shulman, *Arch. Biochem. Biophys.*, **44**, 230 (1953).

(10) V. L. Koenig and J. D. Perrings, *ibid.*, **41**, 367 (1952).

(11) R. L. Baldwin, P. M. Laughton and R. A. Alberty, *J. Phys. Colloid Chem.*, **55**, 111 (1951).

(12) G. E. Perlmann, W. W. L. Glenn and D. Kaufman, *J. Clin. Invest.*, **22**, 627 (1943).

(13) A. Abrams and P. P. Cohen, *J. Biol. Chem.*, **177**, 439 (1949).

Summary.—A constituent has been isolated from cytoplasmic extract of bovine palatine tonsils which possesses chemical, physical and serological characteristics of bovine serum albumin. This component amounts to about 5% of the extract and represents a major component in extracts of this lymphatic organ.

RHEUMATIC FEVER RES. INST.
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Biosynthesis of Penicillin. II.[1] Synthesis of Methionine by a Strain of *Penicillium chrysogenum*

BY MAXWELL GORDON, PAUL NUMEROF AND S. C. PAN

RECEIVED APRIL 15, 1954

Although inorganic sulfate is efficiently utilized in the biosynthesis of penicillin,[2] little is known of the organic sulfur-containing intermediates involved. Numerous sulfur-containing compounds have been added to *Penicillium* fermentations in an effort to improve penicillin yields, but no compounds tested were found to offer advantages over inorganic sulfate.[3] Using techniques of competitive utilization, Stevens, *et al.*,[4] found that *l*-cysteine and *l*-methionine are incorporated by *P. chrysogenum* into penicillin in preference to inorganic sulfate. Arnstein and Grant[5] have demonstrated, using a triple-labeled molecule, that the nitrogen, β-carbon and sulfur atoms of cystine are incorporated with unchanged isotope ratios into the thiazolidine and β-lactam rings of penicillin.

The recent appearance of the reports of Stevens[4] and Arnstein[5] has prompted us to submit this preliminary report. We have found methionine in the broth and in cell extracts and hydrolysates of a penicillin-producing strain of *P. chrysogenum* (Wis 49-133). Methionine is, in fact, produced in yields which make this fermentation eminently suitable for the biosynthesis of S-35 labeled methionine. To our knowledge methionine has not previously been identified as a constitutent of *P. chrysogenum* cells or broth.[6,7] It is of interest that methionine is produced by this organism, under these conditions,[8] as a major metabolite of the precursor inorganic sulfate. Even more striking is the ease with which this methionine can be removed from the cells. Merely heating the mycelial suspension in water

(1) The first communication in this series appeared in *Science*, **118**, 43 (1953).

(2) S. Rowlands, D. Rowley and E. Lester Smith, *J. Chem. Soc.*, S405 (1949).

(3) H. T. Clarke, "Chemistry of Penicillin," p. 666.

(4) C. M. Stevens, F. Vohra, E. Inamine and O. A. Roholt, *Federation Proc.*, **12**, 275 (1953).

(5) H. R. V. Arnstein and P. T. Grant, *Biochem. J.*, **55**, v (1953).

(6) Y. Yokoyama, *J. Antibiotics (Japan)*, **4**, 95 (1951).

(7) P. L. Narasimha Rao and R. Venkataraman, *Experientia*, **8**, 351 (1952).

(8) One hundred ml. of the synthetic medium of F. V. Soltero and M. J. Johnson (*Applied Microbiology*, **1**, 52 (1953)) was used in each flask, except that the ammonium sulfate was omitted in order to obtain material of higher specific activity. The ammonium sulfate was replaced by ammonium nitrate or ammonium chloride, the former giving higher yields of penicillin. The medium contained about 4 mg. of S and gave about 6 mg. of methionine at the end of the fermentation. Washed mycelia were used as the inoculum. The flasks were incubated at 25° for 5 days on a rotary shaker running at 280 r.p.m. In a typical experiment 5 millicuries of S-35 was added per 100 ml. of medium.