TABLE I

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

	BSA	BTA
$E^{1\%}_{280}$ [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).

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(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.

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

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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.

TABLE I

METHIONINE YIELDS FROM PENICILLIUM CELLS

Expt. no.	Wt. of cells extracted, mg.	Vol. of H_2O used, ml.	Wt. of cold methionine added, mg.	Wt. of solids[c] extracted from cells, mg.	Activity of extract, μc.	% of cell activity in extract	Sp. act. of methionine, d.p.m./mg.	% of starting activity in methionine
1	100	5	100	..	..	..	426,000	19
2	400	25	100	137	198[b]	71	1,500,000	17.5
3	1000	25	250	376	404	58	2,160,000	24.5[d]
4	727	25	None	251	168	33	.....	...
5	946	50[a]	153	305	305	45	1,625,000	8.3

[a] 80% Ethanol–water (v./v.) was used for this extraction. [b] Two additional water extractions removed 12.5 and 5.8 μc. in that order. [c] These solids contain about 6 mg. of methionine and traces of other ninhydrin reacting substances; most of the weight is accounted for by salts and undetermined organic constituents. [d] Further extraction of cells gave another 100 μc. of activity which raises the potential radioactive yield of methionine to 30%.

will release methionine in amounts which represent a 30% conversion from the precursor sulfur-35. Since the conversion of sulfate-sulfur to penicillin itself is of the order of 20–30%, it can be seen that the role played by methionine in sulfate metabolism must be a large one.

As cited earlier, cystine has been shown to be incorporated, probably as a single unit, into penicillin.[5] Conversion of methionine to cysteine by way of cystathionine has been demonstrated by Rachele, *et al.*,[9] in the rat. Horowitz[10] has found the reverse reaction in *Neurospora*, and Lampen, *et al.*, have found evidence for methionine–cysteine conversions in both directions using *E. coli* mutants.[11] Hence, the conversion of inorganic sulfur to penicillin and methionine in approximately equal amounts, may suggest a role for methionine in penicillin biosynthesis.

Williams and Dawson,[12] and Wood and Mills[13] have prepared radioactive methionine by the hydrolysis of yeast protein, followed by a number of other manipulations. In our preparation, aqueous extraction of the mycelium followed by a single crystallization with added carrier gives methionine with a radiopurity of >99% in yields, based on starting radiosulfate, of up to 30%. These yields represent a four-fold to thirty-fold increase over those obtained from yeast biosynthesis.[12,13]

Experimental

The data from several experiments are summarized in Table I.

It may be seen from Table I that omitting carrier methionine during the mycelium extraction step reduces the amount of radioactivity removed from the cells (column 7). Hence, the methionine is probably reversibly bound to the mycelium and can be displaced by added methionine. Aqueous ethanol (exp. 5, Table I) is an unsatisfactory solvent for this step. Most of the radioactivity in the aqueous extract is in the form of methionine, the balance being present mainly as inorganic sulfate. Only traces of cysteine have been detected in these extracts.

The methionine was initially observed on autoradiographs of two-dimensional paper chromatograms[14] of mycelial extracts. Addition of carrier methionine to the extracts resulted in exact correspondence of the position and shape of the ninhydrin spots on paper due to carrier and the autoradiographic spots on X-ray film. As additional evidence, the methionine sulfoxide artifact always found on paper chromatograms[15] of methionine likewise duplicated its counterpart on film. Crystallization of the radioactive methionine plus carrier from 80% ethanol (v./v.) gave a product whose specific activity did not change on repeated crystallization. Rechromatographing this crystalline material likewise failed to show any separation of radioactivity and methionine ninhydrin color. Conversion of the crystalline methionine to the crystalline benzoate and acetate gave no change in the molar specific activity. Oxidation of the methionine to the sulfone with H_2O_2 by the method of Dent[15] gave a product whose chromatogram and autoradiograph showed exact coincidence of methionine sulfone spots. Thus, methionine, its sulfoxide, and the sulfone have been demonstrated to be radioactive in these experiments.

Anal. Calcd. for methionine, $C_5H_{11}O_2NS$: S, 21.4. Found: S, 21.3.

Preliminary experiments have indicated that carrier-free methionine of very high specific activity could be isolated from cell extracts by a combination of charcoal[16] and ion-exchange resin chromatography.[17]

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(11) J. O. Lampen, R. R. Roepke and M. J. Jones, *Arch. Biochem.*, **13**, 55 (1947).

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16-Substituted Steroids. X. Androstan-17β-ol-3,16-dione

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Lieberman, Praetz, Humphries and Dobriner[1] have recently established that oxygenation at C_{16} is a general pattern in the metabolism of steroids.

In our syntheses of 16-oxygenated steroids we have had occasion to prepare the compound androstan-17β-ol-3,16-dione, which is of considerable interest as a possible catabolite of male sex hormone in the human organism.[2]

In the present synthesis it was preferred to use as starting material an androstan-17-one with a preformed carbonyl at C_3, for the 16-keto-17-hydroxy-steroid is quite labile to oxidizing agents. Thus, androstane-3,17-dione was protected at C_3 by formation of the 3-diethyl ketal, and this derivative was nitrosated and then reduced following our established procedures. During the last step the 3-diethyl ketal is simultaneously hydrolyzed, yielding as the end-product the free androstan-17β-ol-3,16-dione.

(1) S. Lieberman, B. Praetz, P. Humphries and K. Dobriner, *J. Biol. Chem.*, **204**, 491 (1953).

(2) Also of interest in this connection are our compounds androstane-3α,17β-diol-16-one and androstane-3β,17β-diol-16-one, previously described (M. N. Huffman and M. H. Lott, *J. Biol. Chem.*, **207**, 431 (1954)).