TABLE III. Pyruvate Metabolism After Dialysis of Enzyme Preparation.

Substrate	Oxygen utilized, μatoms	Pyruvate utilized, μM	Acetate formed, μM	CO_2 formed, μM
20 μM pyruvate	19.5	20.1	20.2	17.6
+ 5 μM fumarate	72.3*			

Complete system: see Table I.
* Fumarate blank subtracted.

the CM preparation, even in absence of malonate, converts pyruvate to acetate (Table III).

A comparable situation exists in the CM system of rat liver where it has been shown by Lehninger(10) that pyruvate is either oxidized directly to CO_2 and H_2O, or in absence of condensing partner (*e.g.* in presence of malonate), is converted quantitatively to acetoacetate. The C_2 fragment, if it is not condensed with oxalacetate, can condense with another C_2 fragment to form acetoacetate. This multiple pathway for pyruvate in liver has been studied extensively by Recknagel and Potter(11).

Discussion. The oxidation of pyruvate by the pyruvic oxidase of animal tissues has been shown by Jagannathan and Schweet(12) to lead to the stage of acetyl CoA, since the product is capable of acetylating sulfanilamide. Acetyl CoA can either condense with oxalacetate to form citrate, as has been shown by Ochoa and Stern(2), or it can be decomposed into acetate and CoA, as shown by Gergely and Hele(13). The latter deacylation reaction is so much slower than the former, that it plays a negligible role except when the condensative reaction is blocked.

Summary. Pyruvate is oxidized to CO_2 and H_2O by the cyclophorase-mitochondrial system of rabbit heart. Under appropriate conditions, *e.g.* by blocking with malonate or by prolonged dialysis of the preparation, pyruvate is oxidized to acetate and carbon dioxide, and the C_2 unit formed does not enter the citric acid cycle by condensation with oxalacetate.

We wish to thank Dr. D. E. Green for his guidance and helpful suggestions.

1. Green, D. E., Loomis, W. F., and Auerbach, V. H., *J. Biol. Chem.*, 1948, v172, 389.
2. Stern, J. R., and Ochoa, S., *J. Biol. Chem.*, 1951, v191, 161.
3. Ochoa, S., *Physiol. Rev.*, 1951, v31, 56.
4. Ochoa, S., *J. Biol. Chem.*, 1944, v155, 87.
5. Schweet, R., in a "Symposium on Phosphorous Metabolism," Vol. I, Johns Hopkins University Press, 1951.
6,7. Two preceding papers, XXII and XXIII of this series, PROC. SOC. EXP. BIOL. AND MED., 1952, v79, 349, 352.
8. Friedemann, T. E., and Haugen, G. E., *J. Biol. Chem.*, 1943, v147, 415.
9. Black, S., *Arch. Biochem.*, 1947, v23, 347.
10. Lehninger, A., *J. Biol. Chem.*, 1946, v164, 291.
11. Recknagel, R. O., and Potter, V. R., *J. Biol. Chem.*, 1951, v191, 263.
12. Jagannathan, V., and Schweet, R. S., *Abst. Am. Chem. Soc.*, Sept., Chicago, 1950, v109, 50c.
13. Gergely, J., and Hele, P., in preparation.

Received January 7, 1952. P.S.E.B.M., 1952, v79.

The Origin of Bradykinin. (19377)

C. G. VAN ARMAN.* (Introduced by W. E. Hambourger.)
From the Rheumatic Fever Research Institute, Northwestern University Medical School, Chicago, Ill.

Bradykinin is a hypotensive and smooth-muscle stimulating substance which is formed from the globulin fraction of plasma when acted upon by proteolytic enzymes such as fibrinolysin, trypsin or snake venom. This substance, discovered by Rocha e Silva, Beraldo, and Rosenfeld(1), is characterized as follows: a) it causes a slow contraction of guinea pig, rat, and rabbit intestine which is not prevented by antihistaminics or atropine,

* Present address: G. D. Searle & Co., Chicago, Ill.

b) intravenous injection causes a fall in blood pressure in laboratory animals, c) it is dialysable, stable to heat in acid or neutral solution, and is destroyed by prolonged incubation with trypsin or snake venom, and d) the isolated intestine shows no desensitization after repeated doses. These properties serve to distinguish bradykinin from other gut-contracting substances which may occur in plasma, such as histamine. Bradykinin has been found in the blood of dogs in anaphylactic and peptone shock, but its role is uncertain. In only one of Beraldo's experiments(2) was the amount found sufficient to have been entirely responsible for the fall in blood pressure. The precursor of bradykinin exists only in that portion of the plasma proteins termed the pseudoglobulins, which is precipitated between 35 and 50% saturation with ammonium sulfate(1). According to Neurath *et al.*(3), this includes approximately 21% of the human serum proteins, which when identified by their electrophoretic mobilities are found to be divided as follows, per 100 ml of serum: 0.15 g of albumin, and of the globulins, α_1, 0.12 g; α_2, 0.55 g; β and γ combined, 0.67 g.

It was of interest to determine which of these components contains the precursor. An opportunity to attempt this was afforded by the methods developed for fractionation of plasma proteins by E. J. Cohn and his group at Harvard during recent years. These methods allow sharper separation of the several types of proteins in the native condition than is possible by "salting-out" procedures. Cohn's method No. 10(4) separates the proteins into 6 fractions:

I —Fibrinogen and anti-hemophilic globulin
II + III—γ-globulins (antibodies)
β_1-globulin, including lipoprotein (complement, midpiece, prothrombin)
β_2-globulin (isoagglutinins)
plasminogen (profibrinolysin)
IV —α_1-lipoproteins (carry cholesterol, phospholipids, some steroids)
α_2-glycoproteins, α_2-mucoproteins } (hypertensinogen, cholinesterase, also carries bilirubin)
β_1-metal combining protein (thyrotropic hormone, iodoprotein)
phosphatase
V —Serum albumin
VI —traces of IV and V
α_1-small acid protein
α_2-protein
β_1-protein
other small proteins and peptides
uric acid

These fractions may be further subdivided. The plan of the present experiments was to test each of the 6 main fractions for yield of bradykinin, and then to identify the precursor, so far as possible, by testing the various subfractions.

Methods. Bradykinin was made in 5 batches by treatment of 1-liter portions of bovine pseudoglobulin solutions with snake venom as described by Rocha e Silva *et al.*(1). Potencies measured on the isolated ileum varied considerably. The best preparations were further purified according to the advice of Dr. W. T. Beraldo as follows: Some 600 mg of crude material obtained from 1 liter of bovine plasma were extracted by stirring 3 times with 5 cc portions of glacial acetic acid, and the residue was discarded. Eight volumes of ether were added to the acetic acid solution, and the precipitate was centrifuged down, washed twice with ether, twice with acetone, and dried at 40°C. This material apparently lost no potency during storage in the desiccator for 2 months. Assays were performed with the usual isolated guinea pig ileum in oxygenated Tyrode's solution, atropinized and usually treated with diphenhydramine. Whenever snake venom was used, the ileum was previously densensitized to this agent by repeated doses until no response was obtained.

Experiments and results. The several bovine fractions, separated according to Cohn's method 5(5) were kindly supplied by Dr. James B. Lesh of Armour Laboratories, Chicago, Ill. Method 5 is essentially the same as 10, but the main difference, so far as this study is concerned, would seem to be that Fraction IV is obtained in a more impure state by the older method. Suspensions of each fraction were made in Tyrode's solution at pH 7.6 and centrifuged. One cc of the supernatant solution was incubated at 37°C for 90 seconds with 60 μg (0.06 cc) of a solution of powdered venom from Bothrops jararaca,† and 0.1 cc portions were tested at once on the isolated

ileum. Fractions I, II + III, V, and VI in 5% suspension yielded no bradykinin, or any other smooth muscle stimulating substance, even upon longer periods of incubation (Fractions III-0 and III-1 yielded a relatively insignificant amount from 5% suspensions). Practically the entire activity of the plasma was contained in Fraction IV, tested as a 1% suspension. The 2 subfractions of IV, termed IV-1 and IV-4, were both very active in 1% suspension. IV-4 yielded approximately twice as much bradykinin as IV-1 (both subfractions had been made from the same lot of plasma). The insoluble part of these fractions did not contain any precursor.

Since narrower subfractions of IV were not available, an attempt was made by other means to discover which component of IV contained the bradykinin precursor. The plan was to find which of the several electrophoretic components would be destroyed or reduced in amount by incubation with snake venom. A 2% suspension was centrifuged at slow speed and the sediment discarded. The solution was dialyzed against distilled water at 2°C for 72 hours and against barbital buffer of pH 8.54 for 24 hours. It was then centrifuged at 19,000 rpm for 45 minutes. A creamy fat layer rose to the top. The solution underneath was still murky but was used nevertheless. It was subjected to electrophoresis‡ in a Tiselius apparatus with a Philpot-Svensson schlieren optical system, at 0.4°C for 128 minutes in barbital buffer of pH 8.54, conductivity 0.00312 $ohm^{-1}cm^{-1}$, and ionic strength 0.1, in a field of 6.50 volts/cm. Three peaks were found, corresponding in mobilities to albumin, α- and β-globulins. The relative amounts were estimated by magnification of the photographic curves, with planimetric measurement of the areas under the 3 peaks, which were found to constitute 35, 33, and 32% of the total area, in that order. Sixteen cc of a similar solution of IV, centrifuged at high speed, were then treated with 1.6 mg of snake venom at 42°C for 2 minutes and rapidly cooled, with the object of destroying the bradykinin precursor. After dialysis for 4 days at 2°C as above and further high speed centrifugation, the solution was submitted to electrophoresis under the same conditions. The resulting photographs revealed that the albumin, α- and β-globulins constituted 39, 35, and 25% of the total. This result is not taken to be significantly different from the control, in view of the errors inherent in the technic. There is a slight indication, however, that the β component was reduced more than the others.

Discussion. Fraction IV in Cohn's method 10 comprises approximately 9% of the human plasma proteins(4). It is subdivided into "extract IV-6 + 7", which accounts for 6%, and IV-1, which accounts for 3% of the plasma proteins. It is to be emphasized that bovine and human plasma are different, and with identical treatment yield fractions of somewhat different composition. Using method 5(5), Lesh found that of a final yield of 56 g of dry protein obtained per liter of bovine plasma, Fraction IV-1 constituted 5 g, or 9%, while IV-4 constituted 3 g, or 5%. When made from human plasma, IV-1 contains mostly α-globulin, much of it a lipoprotein containing about 35% lipid, some β-globulin and albumin(6). IV-4 contains nearly lipid-free α- and β-globulins, with some albumin. It contains serum esterase and a specific iron-binding globulin.

Hypertensinogen is present in high concentration in the supernatant after precipitation of Fraction IV-1; however, it is largely inactivated upon precipitation of IV-4 from solution. IV-4 may be further fractionated into IV-5, 6 and 7; IV-6 contains the hypertensinogen(6). The possible physiological reciprocal relationship between hypertensin and bradykinin has been proposed by Rocha e Silva(7). It is of some interest that these two opposing factors, one hypertensive, the other hypotensive, have their origin in a common fraction of the plasma proteins.

The precursor is probably not in the albumin contaminants of IV-4, since Fraction V (albumin) gave no response (this agrees with observations of Rocha e Silva *et al.*). Since

† Kindly furnished by Dr. W. T. Beraldo. Original source: Butantan Institute, Sao Paulo, Brazil.

‡ In this work I was fortunate to have the advice and guidance of Dr. Eugene L. Hess.

IV-4 gives a better yield than IV-1, and since the α-globulins are present in lesser amounts in IV-4 than in IV-1, one may suspect that the α-globulins are not responsible; contrariwise, the β_1-globulins are present in greater amount in IV-4, and could be suspected of containing the precursor. The electrophoretic experiment above, although not decisive, points to the β-globulins.

Summary. 1. The precursor of bradykinin is contained in Fraction IV of the bovine plasma proteins (Cohn's nomenclature). 2. Of the 2 main subfractions, IV-4 yields approximately twice as much bradykinin as IV-1.

I wish to thank Dr. C. A. Dragstedt for advice in preparation of the manuscript.

1. Rocha e Silva, M., Beraldo, W. T., and Rosenfeld, G., *Am. J. Physiol.*, 1949, v156, 261.

2. Beraldo, W. T., *Am. J. Physiol.*, 1950, v163, 283.

3. Neurath, H., Volkin, E., Erickson, J. O., Craig, H. W., Putnam, F. W., and Cooper, G. R., *Am. J. Syphilis, Gonorrhea and Venereal Dis.*, 1947, v31, 347.

4. Cohn, E. J., Gurd, F. R. N., Surgenor, D. M., Barnes, B. A., Brown, R. K., Derouaux, G., Gillespie, J. M., Kahnt, F. W., Lever, W. F., Liu, C. H., Mittelman, D., Mouton, R. F., Schmid, K., and Uroma, E., *J. Am. Chem. Soc.*, 1950, v72, 465.

5. Cohn, E. J., Strong, L. E., Hughes, W. L., Jr., Mulford, D. J., Ashworth, J. N., Melin, M., and Taylor, H. L., *J. Am. Chem. Soc.*, 1946, v68, 459.

6. Edsall, J. T., *Advances in Protein Chemistry*, 1947, v3, 383.

7. Rocha e Silva, M., *Bradicinina.* Thesis, Faculty of Medicine of the University of Brazil, Sao Paulo, 1951.

Received January 9, 1952. P.S.E.B.M., 1952, v79.

Cross-Resistance Studies with Streptomycin, Streptothricin, Neomycin, and Streptolin. (19378)

JOSEPH F. PAGANO, MARVIN J. WEINSTEIN, AND RICHARD DONOVICK.

From the Squibb Institute for Medical Research, New Brunswick, N. J.

Several studies have been reported concerning the cross-resistance of various bacterial species with streptomycin and streptothricin (1-4) and neomycin(5-8). In the present study, cross-resistance has been found among this chemically and biologically related group of antibiotics, but not with every organism studied.

Methods. Strains of *Escherichia coli* (D-56), *Streptococcus fecalis* (ATCC-9790), *Pseudomonas aeruginosa* (D-163), *Micrococcus pyogenes var. aureus* (209P), *Salmonella schottmulleri* (D-51), *Bodenheimer's bacillus* (D-78) and one strain of *Escherichia coli* previously made resistant to chloromycetin, aureomycin, and terramycin, designated *E. coli-R* (D-56-9), were used in this study. Several of these organisms were made resistant to either streptomycin hydrochloride (Squibb, 808 u/mg), streptothricin hydrochloride (Merck, 500 u/mg) or neomycin sulfate (Squibb, 175 u/mg) by repeated exposure to increasing amounts of the antibiotic in yeast beef broth medium (Difco, pH 6.8). Cultures were incubated at 37°C for 1-3 days. The minimal inhibiting concentrations (MIC) of antibiotic were determined by the agar dilution method, the organism being streaked on a series of yeast beef agar plates containing increasing concentrations of the antibiotic. Prior to streaking, the cultures were centrifuged for 30 minutes at 2000 RPM, washed and resuspended in saline. It was noted that the MIC in broth cultures was usually lower than the MIC observed on agar plates. To determine the degree of resistance and cross-resistance developed, an organism made resistant to one of the antibiotics was simultaneously streaked on agar plates containing the other antibiotics. Two-fold increases in concentration of antibiotic were utilized. When the organism grew well at one level and was inhibited completely by the next higher level of antibiotic, the MIC was considered as the mid-point between the two levels. Repeat testing of cross-resistance indicates that the variation