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rest and under physical and emotional stresses. Hence, the observed effects of insulin and electroconvulsive therapies upon uropepsin excretion assume considerable theoretical significance.

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Biosynthesis of the C^{14}-Labeled Form of Dextran[1]

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Dextran is a polysaccharide, made up solely of glucose units, produced by bacterial fermentation of sucrose. Only the glucose portion of the sucrose molecule is utilized in the biosynthetic process. Dextran of suitable molecular size and purity is currently of interest as a synthetic plasma volume expander, particularly for use in event of large-scale catastrophe in which natural blood plasma supplies might be limited. Dextran has been tested clinically with success, but its metabolic fate in the body is inadequately known, since the best analytical procedures account for only about half of injected dextran. It was concluded that these metabolic questions could best be resolved through the use of an isotopically labeled form of dextran.[4]

Argonne National Laboratory and Commercial Solvents Corporation, at the request of the Office of the Surgeon General of the U. S. Army, and under the direction of the Subcommittee on Shock, National Research Council, cooperated in biosynthesizing C^{14}-labeled dextran at two different levels of activity, one designed for use in animal experiments, the other for human clinical experiments. Since 1949 the Research and Development Laboratories, Commercial Solvents Corporation, have conducted a dextran research program and at present have developed a successful clinical grade of dextran. The present report outlines the experimental procedures and results incident to the successful completion of the biosynthesis program.

Essentially, the problem involved the biosynthesis of labeled C^{14} sucrose, followed by the biosynthesis of labeled dextran through fermentation of this sugar. The quantity and absolute activity of labeled dextran required for proposed laboratory and clinical experiments necessitated the handling of approximately 0.3 c of radiocarbon. In order to determine the adequacy of both proposed experimental equipment and procedures, a small quantity of low absolute activity C^{14} dextran was biosynthesized. As soon as these studies were evaluated large-scale biosyntheses were initiated.

C^{14} sucrose was biosynthesized by allowing carbohydrate-depleted, excised *Canna* leaves to photosynthesize in the presence of $C^{14}O_2$ in a 38-liter hermetically sealed, leaf-chamber system.[5] This plant was selected because of its reported high efficiency in converting $C^{14}O_2$ to sucrose during photosynthesis (*1*). A total of 309.7 mc of $C^{14}O_2$ was generated from $BaC^{14}O_3$ in eight experiments employing a total of 30 leaves, weighing 208.8 g fresh. The separate photosynthesis periods varied from 6 to 24 hr and resulted in fixation of 308.0 mc in the leaves, or 99.4% of that generated.

A total of 175.2 mc of C^{14} sucrose was biosynthe-

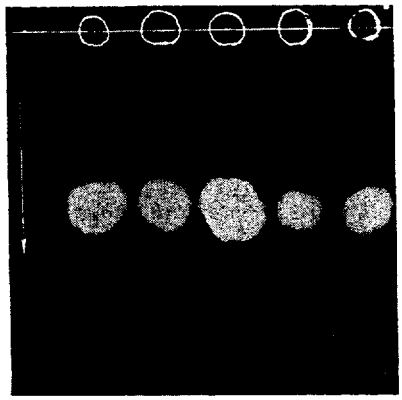

FIG. 1. Radioautograph of paper chromatogram of five separate lots of C^{14} sucrose showing their lack of contamination. Paper partition chromatogrammed for 48 hr at 20° C with BuOH : EtOH : H_2O (45 : 5 : 50) as irrigating solvent. Radioautographed for 30 days using Eastman No-screen x-ray film.

[1] The experimental work was carried out in the Division of Biological and Medical Research, Argonne National Laboratory, under Contract No. DA-49-007-MD-102 between Commercial Solvents Corporation and the Office of the Surgeon General of the U. S. Army. The authors are greatly indebted to Weldon Brown, University of Chicago, and to F. H. Schultz, Jr., Commercial Solvents Corporation, for their interest, encouragement, and counsel. They also acknowledge the able assistance of Lt. Col. E. J. Pulaski, Army Medical Center; Phillip H. Abelson, Carnegie Institution; Walter L. Bloom, Emory University, and A. M. Brues, Argonne National Laboratory, in planning various phases of this project.

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[3] Commercial Solvents Corporation, Terre Haute, Indiana. The following Commercial Solvents Corporation personnel also assisted with various phases of the program: Robert Cundiff, L. R. Jones, and Dona Graam.

[4] The metabolism studies are being conducted by various investigators, at the direction of the Subcommittee on Shock, National Research Council.

[5] The detailed experimental methods utilized for the biosynthesis and isolation of C^{14} sucrose and other plant fractions from Canna leaves are to be presented elsewhere.

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sized. A total of 741.8 g of pure carrier sucrose was added to the isolated carbohydrate leaf extracts, utilizing the isotope dilution technique, and 684.9 g of crystalline sucrose containing 168.4 mc was obtained. The average activity was 245.9 μc/g of sucrose, and the average sucrose extraction efficiency was 96.1%. On the basis of the total quantity of C^{14} fixed in photosynthesis, the over-all efficiency in conversion into sucrose was 56.9%. With certain individual leaf biosynthesis runs, the sucrose efficiency was as high as 72.1%. Fig. 1 shows a radioautograph of a paper chromatogram of five separate lots of crystalline C^{14} sucrose and indicates the lack of contamination. Two of the five lots of sucrose were degraded, using enzymatic and microbiological methods (*2*), and were found to be uniformly labeled.[6]

Identical fermentation techniques were employed for the biosynthesis of dextran at each of the two different activity levels—namely, 106.8 μc/g for animal experimentation and 5.68 μc/g for use in human beings. The bacterium *Leuconostoc mesenteroides* was employed in these fermentations. The medium used was that described by Jeanes, Wilham, and Miers (*3*). Fermentations were conducted in 1-, 4-, or 6-liter flasks, containing 300, 3000, or 4000 ml of medium, respectively, sterilized at 116° C for 30 min. Small inoculum cultures used for seeding large-scale C^{14} fermentation flasks were prepared with C^{14}-labeled sucrose, and the entire contents of the culture flask were added equally to each fermentation flask. The fermentation period was terminated during the 26th hour.

Because of the large volumes involved, a total of seven fermentation runs was necessary, five for the low activity level dextran and two for the high activity level material. The native dextran was precipitated from the fermentation beer with methanol. The crude dextran was dissolved in water and reprecipitated with methanol. This was repeated several times more for purification.

For depolymerization the native dextran was subjected to acid hydrolysis, the course of which was followed by determination of viscosity change. The hydrolysates were cooled rapidly, made slightly alkaline with sodium hydroxide, and filtered through a layer of filter aid. The hydrolyzed dextran was fractionated and refractionated from aqueous solution by the addition of methanol, separating the material into three fractions: high molecular weight, intermediate (clinical), and low molecular weight. Wherever necessary the determination of dextran in solution was carried out by the anthrone method (*4*).

In the preparation of the dextran for animal experimentation 303 g of C^{14} sucrose having an absolute activity of 114.7 μc/g was fermented. The clinical fractions from two hydrolyses were reprecipitated together from aqueous solution by the addition of methanol. The dextran was dried, ground to 40 mesh in a Wiley mill and packaged in 1.0 g units. The yield of dry, pyrogen-free dextran of clinical size was 25.3 g containing 2.70 mc of C^{14}. The specifications of the final product are listed in Table 1.

TABLE 1

SPECIFICATIONS OF C^{14} DEXTRAN FOR ANIMAL AND CLINICAL EXPERIMENTS

Characteristic	Dried dextran for animal use	Dextran in solution for human use
Sterility to yeasts, molds, and bacteria	—	Passed
Average dextran concentration, g/100 ml	—	6.06
Viscosity of a 6% solution at 25° C (centistokes)	3.25	2.79
Intrinsic viscosity at 25° C	—	0.20
Mol weight (wt av by light scattering)	126,000*	82,600*
Nitrogen, mg/100 ml	—	0.56
pH	—	5.55
μc/g on solid	106.8†	5.68‡
μc/g based on carbon recovered in combustion	116.8†	6.35‡

* Determined by E. E. Toops, Jr., Commercial Solvents Corporation.
† Assayed by Alexander Van Dyken, Chemistry Division, Argonne National Laboratory.
‡ Assayed by K. E. Wilzbach, Chemistry Division, Argonne National Laboratory.

For the preparation of dextran for human experiments, purified native dextran obtained from the fermentation of 17,800 g of C^{14} sucrose (5.99 μc/g) was hydrolyzed in two batches. Hydrolyses were carried out in twin circular white-enameled laundry tubs, insulated externally, and fitted with glass coils for steam heating and water cooling. All exposed metallic surfaces were coated with Tygon plastic, and the tubs were covered during the hydrolysis with Tygon plastic sheeting. Hydrolysates were pumped through Tygon tubing by means of a glass-lined centrifugal pump into a ceramic filter (diameter, 3 ft) and filtered by suction through a layer of filter aid. Fractionation was carried out as noted above. The final sterile and pyrogen-free product was made up to a 6% dextran solution to which 0.9% sodium chloride had been added. It was bottled in 500-ml units. A total yield of 68 bottles containing 12.58 mc was obtained. The specifications of the final product are listed in Table 1.

Radioactive dextran has been placed in the hands of a number of investigators.[7] Preliminary results definitely indicate that dextran is metabolized by mice, rats, dogs, and human beings, with a substantial portion of the labeled dextran appearing in the expired air as $C^{14}O_2$. One preliminary report has already

[6] The authors wish to thank Martin Gibbs, Brookhaven National Laboratory, Upton, Long Island, N. Y., for conducting the degradation studies.

[7] J. Garrott Allen, University of Chicago Medical School; Walter L. Bloom, Emory University Medical School; Leon Hellman, Sloan-Kettering Institute; Joe Howland and Rodger Terry, School of Medicine and Dentistry, University of Rochester; Surgical Research Unit, Brooke Army Hospital, Fort Sam Houston, Texas; and Harry M. Vars, School of Medicine, University of Pennsylvania.

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been published (*5*). Detailed publications will be forthcoming from the various investigators and will appear elsewhere.

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A Dominant, Sex-Linked Mutation in the House Mouse

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Evidence for sex-linked mutations in the mouse has been presented by Hauschka *et al.* (*1*), Chase (*2*), Bittner (*3*), and Strong (*4*). Only one of these reports (*1*), however, involved a morphological mutation. The results of matings of a new dominant mutation in the mouse, Bent-tail (Bn), suggest that this mutation may be sex-linked.

The expression of Bent-tail in the female may range from a single, almost imperceptible kink to a series of well-defined kinks. In some extreme cases, the tail is bent back on itself and may be pressed against the body. In females, the tail is usually normal in length; in males, however, the tail is usually half the normal length, and the kinks are generally restricted to the end of the tail. Except for the kinks and shortness, the tail is apparently normal.

A single Bent-tail male was found in a litter of 7 mice resulting from a mating between a normal female of the Namru strain (*5*) and a bald, hr^{ba}, male (*6*).

TABLE 1

A SUMMARY OF MATINGS OF BENT-TAIL AND NORMAL MICE

Parents		Offspring				
		Bent-tail		Normal		No. of litters
Female (phenotype)	Male (phenotype)	Female	Male	Female	Male	
24-A (+)	24-1 (Bn)	23	0	2*	13	4
24-A (+)	Namru (+)	0	0	7	4	1
Namru (+)	24-1 (Bn)	4	0	0	2	1
Namru (+)	24-1 (Bn)	3	0	0	6	1
Namru (+)	24-1 (Bn)	3	0	0	4	1
130 (+)*	Namru (+)	2	1	3	1	1
131 (+)*	Namru (+)	1	1	3	4	1

* Phenotypically normal, genotypically Bent-tail.

[1] This work was supported in part by a contract between the University of California, Department of Bacteriology, and the Office of Naval Research. The opinions contained in this report are not to be construed as reflecting the views of the Navy Department or the Naval service at large.

This male, 24-1, mated with a normal sib, 24-A, produced Bent-tail females and normal males, suggesting that the mutation was dominant and apparently sex-linked. Two females could not be positively identified as Bent-tail because of the poorly defined single kink. These 2 females when outcrossed to normal Namru males yielded Bent-tail and normal mice. When the original normal female, 24-A, was outcrossed to a normal Namru male, only normal mice resulted. Finally, the original Bent-tail male, 24-1, was outcrossed to 3 normal Namru females. These matings also indicated that the mutation was sex-linked and dominant. The results of all these matings are summarized in Table 1.

The aberrant sex ratio in litters from the original matings between 24-A and 24-1 has been interpreted as being due to a lethal gene in one of the sex chromosomes of the female (*1*). Matings are in progress to test the validity of this hypothesis. Additional matings have been initiated to determine whether homozygous Bent-tail females occur.

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Uraninite from the Grey Dawn Mine, San Juan County, Utah

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Massive chunks of primary uraninite have been found intimately associated with carnotite-bearing ores from the Grey Dawn Mine located on a small tributary of La Sal Creek near the southeast flank of the La Sal Mountains, San Juan County, Utah. This spectacular occurrence of uraninite was brought to the attention of the writer by Ace Turner, operator of the Grey Dawn Mine, when he reported uncovering approximately a thousand pounds of some black ore, "heavy as lead," and assaying about 64% U_3O_8.

Although uraninite has been recognized by Gruner (*1*), Kerr (*2*), and others (*3*) in other sedimentary and igneous rocks on the Colorado Plateau, this discovery is believed the first in carnotite ores from the Salt Wash sandstone member of the Morrison formation, which currently contributes the major proportion of our domestic production. Its presence in these ores may modify the present concept of the origin of the carnotite-bearing sandstone deposits of the Colorado Plateau as interpreted by Fischer (*4*).

A field examination of the mine revealed three pod-like occurrences of uraninite-bearing ore in separate

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parts of the mine. The largest pod occurred as a mass 18″–24″ thick by 4′ wide and 10′ long. The massive uraninite, 3″–5″ thick, rested on 6″–8″ of black vanadium ore commonly designated as corvusite. This was followed by 8 or more inches of a yellow carnotite-type ore, which was topped by 6″ of powdery red hewettite.

The specimens of uraninite-bearing ore that have been collected show a black central core of uraninite, in part laced with delicate seams of yellow, orange, and green uranium minerals, and the whole surrounded by carnotite. Although some of these minerals have been tentatively identified, there are many others that have not.

The Bureau of Mines at Salt Lake City, Utah, made a preliminary microscopic and chemical examination of one specimen in August 1951. It consisted of half black oxide and half yellow alteration products. The chemical analysis of the material as a whole gave 66% U_3O_8, 4.8% SiO_2, 7.7% H_2O, and 0.95% copper. There was considerable vanadium present, as well as a minor amount of arsenic. Regarding the identity of the minerals, Harold L. Gibbs states:

> The minerals tentatively identified in this small rich sample are uraninite, beta-uranotil, carnotite, and gummite. An X-ray photograph of the black uranium oxide by Allen King of the University of Utah gave the lines of uraninite. The beta-uranotil was observed as thin 100 plates with parallel extinction, gamma = 1.685, and strong pleochroism, yellow to colorless. Grains in cold concentrated HCl leave a silica residue. Intergrown with the beta-uranotil in all cases was an extremely fine yellow mineral, index more than 1.74, tentatively identified as carnotite because of the presence of vanadium in the same grain. Two yellow isotropic minerals, one of low index near 1.60 and one near 1.73 are tentatively identified as gummite. The nature of the copper and arsenic occurrence was not identified.

Some of these minerals are new to the mineralogy of the carnotite deposits, and it is expected that others will be found and identified by the U. S. Geological Survey following examination of other specimens from this deposit. When the USGS studies have been completed it is believed that there also will be more information on the nature of the uraninite and its alteration products.

The Grey Dawn carnotite deposit is similar in many respects to other carnotite deposits of the Salt Wash sandstone except for the presence of uraninite, gummite, and β-uranotil. It has individual ore pods that are lenticular, irregular in shape, and essentially parallel to the gentle dip of the sandstone bed. Tiny northwest-trending fractures cross the favorable bed in the vicinity of the ore bodies and are believed to be one of the structures influencing the deposition of uraninite. Although there is no surface indication of the Grey Dawn deposit at the rim outcrop, nevertheless some 120′ back of the rim a combination of favorable host rock and structure resulted in forming an ore body.

In the carnotite deposits of the Salt Wash sandstone, no original mineral has ever been found the alteration of which could produce carnotite. The discovery of massive uraninite in the Grey Dawn Mine suggests a source for the uranium, and an unidentified black mineral high in vanadium collected here, as well as in other mines, suggests a source for the vanadium. Not only does the uraninite offer a solution to the origin of carnotite, but it supports the theory that the carnotite-bearing deposits are the product of the alteration of ores of hydrothermal origin. Evidence connecting the deposits with igneous activity is not conclusive, but the presence of the laccolithic La Sal Mountains near the Grey Dawn Mine suggests a close association. The world occurrences of uraninite are listed as associated with heated solutions, and as the Grey Dawn uraninite is not of placer or detrital origin, the possibilities of a hydrothermal origin for the uraninite seem exciting and well worth testing. From the data that are being assembled it appears that a new classification of uraninite-bearing ores is being developed on the Colorado Plateau.

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An Effect of Proteins and Proteoses on the Cellulase of *Myrothecium verrucaria*[1]

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During work on purification of the cellulase of *Myrothecium verrucaria* Alb. et Schw., strain USDA 1334.2, a marked stimulation of cellulase activity by the addition of proteins and proteoses has been observed. Protein effects on enzymes are well known but the present evidence suggests that the mechanism of the effect on cellulase is unusual.

The enzyme preparation ("Fraction 80") on which the effect has been most studied was obtained from a culture filtrate by concentration and repeated fractional precipitation with ammonium sulfate (*1*) and stored as a freeze-dried powder. From subsequent evidence its cellulase content has been estimated to be approximately 40% of the total protein. Enzyme activity was assayed from the micro-Somogyi titer of the reducing sugar formed during incubation of enzyme in 20 ml of assay medium for 17 hr at 35° C, with continuous rotary shaking. The assay medium consisted of a 1% dispersion of cellulose substrate in 0.05 *M* acetate buffer of pH 5.6, with sodium pentachlorophenate as antiseptic. The cellulose substrates have been previously described (*1*): "precipitated cellulose," a finely

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