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2 μM of acid stable (to 1 N HCl at 100° for 3 hr.) organic phosphate are formed per μM of HDP disappearing. Filter paper and ion exchange chromatography indicate this organic phosphate to consist of two components corresponding to authentic PG and 3-PGA. Preliminary results suggest the equimolar formation of these two components.

388. Anaerobic P³² incorporation into acid-soluble phosphates and phospholipid of brain homogenates. J. F. BERRY AND W. C. McMURRAY (introduced by R. J. ROSSITER). Dept. of Biochemistry, Univ. of Western Ontario, London, Canada.

Water homogenates of rat brain suspended in tris buffer, 0.05 M (pH 7.4) and reinforced as described by Reiner (*Arch. Biochem.* 12: 327, 1947) were incubated with 120 μc inorganic P³² for 1 hr. at 37.5°. Portions of the TCA supernatant were used for the separation of HDP, ATP, ADP and AMP on washed Whatman #4 filter paper, by a modification of the procedure of Krebs and Hems (*Biochem. Biophys. Acta* 12: 172, 1953). Specific activity determinations were made on the eluted spots and on purified lipid extracts of the TCA precipitate. Such a system incorporated P³² into all fractions as readily anaerobically as aerobically. The specific activities were in the order: HDP > ATP > ADP > AMP > lipid P. For optimal glycolysis and P³² incorporation, AMP could replace ATP in the medium, provided HDP was present. In contrast to the increase found in the aerobic system, fluoride (0.01 M) caused a decrease in the anaerobic incorporation into all fractions. The aerobic increase can be explained in terms of ATP-ase inhibition, whereas the anaerobic decrease can be explained by the inhibition of one of the glycolytic enzymes, presumably enolase. The inhibitory effect of dinitrophenol (7.5×10^{-3} M), observed aerobically, was much less under anaerobic conditions. In the anaerobic system the incorporation into lipid P always paralleled the incorporation into ATP, which paralleled glycolysis. This suggests that the initial stage in the incorporation into lipid P involves the glycolytic formation of high-energy phosphate compounds.

389. Thioether cleavage. FRANCIS BINKLEY. Dept. of Biochemistry, Emory Univ., Ga.

Cystathionine and lanthionine are cleaved at high rates at physiological temperatures in the presence of traces of metal ion, pyridoxal or pyridoxal phosphate and chelating agents; homo-cysteine and cysteine, respectively, were formed. Zinc ion was found to be most effective for lanthionine and cupric ion was specific for cystathionine. Chelating agents were essential for the cleavage of cystathionine and greatly activated the cleavage

of lanthionine. The action of chelating agents was related to optimal pH values of the reactions; the optimal pH was 9 for lanthionine and was 12 for cystathionine. The simple chelate complexes conformed to the usual kinetic analyses, were inhibited competitively by related compounds and non-competitively by cobalt and mercury ions, were heat labile and demonstrated optical specificity in their action; mesolanthionine was cleaved with the formation of β-cystine. Certain crystalline proteins were found to behave as effective chelate compounds but, in some cases, would not catalyze the reaction to complete cleavage. The catalytic effect of the chelates has been interpreted in the following manner: CHE(ate) - ME(ate) - CO(-enzyme) + S(substrate) = CHE - ME - CO - S → CHE + CO + MEP(react) = CHE - ME - CO + P. Thus, the important considerations are the affinity of the metal for the chelate compound and for the product (the second equilibrium).

390. Carcinogenic activity of cholesterol degradation products. FRITZ BISCHOFF, GUILLERMO LOPEZ,* J. J. RUFF* AND CHARLES L. GRAY.* Santa Barbara College Hosp. Research Inst., Santa Barbara, Calif.

Administered to Marsh-Buffalo mice in sesame oil, an impure progesterone (BISCHOFF AND RUFF, *Cancer Res.* 6: 403, 1946), impure cholesterol, lathosterol, and the non-keto fraction of cholesterol oxidized in soap suspension (BISCHOFF, LOPEZ AND RUFF, *Abstr. Am. Chem. Soc. March, 3C, 1954*) have produced higher incidences of fibrosarcoma than controls which received only sesame oil, or sesame oil plus pure cholesterol, pure progesterone, or cholestenone. Similar carcinogenic results were obtained by Mirand, Reinhard and Goltz (*Proc. Am. Assoc. Cancer Res.* 1, No. 2, 33, 1954) with desoxycorticosterone acetate in sesame oil, negative results with sesame oil alone. The following table shows the highly significant results obtained by us with a number of steroids administered in sesame oil as compared with controls which received only sesame oil. Each group was comprised of 33 mice. Intact males were used for the first 3 groups; castrated females for the last 2 groups.

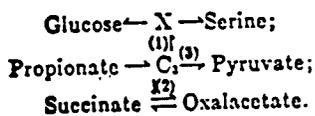
Compound	% fibrosarcoma
None, control	0
6 hydroxy Δ ⁴ cholestene-one	19
Δ ⁴ cholestene-3,6-dione	34
None, control	0
Cholesterol α oxide	43

Conclusion: The combination of sesame oil and certain oxidation products of cholesterol is carcinogenic in mice. (Supported in part by grant C

1586 C from the Natl. Cancer Inst., Natl. Insts. of Health, PHS. Acknowledgment is made to Louis F. Fieser for compounds.)

591. Propionate metabolism in intact dairy cow. A. L. BLACK AND MAX KLEIBER (introduced by H. Goss). *School of Veterinary Medicine and College of Agriculture, Univ. of California, Davis.*

Propionate is glucogenic but the pathway for its metabolism is still undecided. Results obtained with cows on the transfer of C^{14} from fatty acids to amino acids of casein and to lactose cannot be entirely explained on the basis of presently proposed pathways for propionate metabolism. Following injection of 1- C^{14} or 2- C^{14} acetate (I) or butyrate (II), aspartic (III) and glutamic (IV) acids had specific activities 2-6 times higher than serine (V), alanine (VI), or lactose (VII). The specific activities of (V), (VI), and (VII) were approximately equal. After injecting propionate-1- C^{14} and -2- C^{14} , (V) and (VII) were recovered with specific activities approximately equal to those of (III) and (IV). The C_2 units from (I) and (II) presumably label intermediates of the Krebs cycle and then (III) and (IV), via transamination. If the major pathway for propionate metabolism were via succinate in the Krebs cycle, propionate should label (III) and (IV) more highly than (V) and (VII). Our observations, therefore, indicate for propionate pathways other than via the Krebs cycle (succinate). Evidence indicating that pyruvate was not on the main pathway for propionate- C^{14} metabolism resulted from the observation that alanine had lower specific activities than (III), (IV), (V) or (VII). Our results are best explained as follows:



This mechanism is similar to one proposed by Shreeve (*J. Biol. Chem.* 195: 1, 1952). Our results indicate that the C_2 unit is transferred over pathway 1) and 2) with about equal isotope dilution but more slowly (or with greater dilution) over pathway 3) to pyruvate. (Supported by a contract with the Natl. Science Fdn. and the U. S. Atomic Energy Commission.)

592. Enzymatic synthesis of uroporphyrin. LAWRENCE BOGORAD (introduced by S. GRANICK). *Dept. of Botany, Univ. of Chicago, Chicago, Ill.*

The utilization of porphobilinogen (PBG) for the enzymatic synthesis of porphyrins by broken-cell and cell-free preparations of *Chlorella* and certain tissues of some other plants has been re-

ported (BOGORAD AND GRANICK, *Proc. Nat. Acad. Sci.* 39: 1176, 1953). An enzymic preparation now has been made from acetone powder of spinach leaf tissue which catalyzes the synthesis of uroporphyrin I (characterized by paper chromatography) from PBG. Further fractionation of this preparation has been accomplished by zone electrophoresis on starch blocks. One fraction so obtained catalyzes the decamination of PBG which simultaneously disappears from the reaction mixture (as assayed by the Ehrlich *p*-dimethylamino-benzaldehyde procedure) without concomitant appearance of porphyrin. Upon the addition of crude enzyme preparation to reaction mixtures in which the PBG has been exhausted by the action of PBG decaminase, there appears rapidly a compound which has a strong absorption maximum at about 500 $m\mu$. This, in turn, is followed shortly by the appearance of uroporphyrin. The nature of the intermediates is being investigated. The liberation of ammonia from PBG occurs aerobically as well as in an atmosphere of nitrogen in the presence of the decaminase. Ammonia is liberated neither from β -phenylethylamine nor from glutamine when either of these compounds is incubated with PBG decaminase prepared from spinach leaf tissue. (Supported in part by grants from the Natl. Science Fdn. (G618) and from the Natl. Insts. of Health (G-4098).)

593. Some factors affecting measure of potency of botulinum toxin. ALDEN K. BOOR, HUGH B. TRESSLIT* AND EDWARD J. SCHANTZ.* *Camp Detrick, Frederick, Md.*

Variation in the system of dilution of the liquid culture of *Clostridium botulinum* for the mouse assay led to erratic indications of potency. Preparation of a stable stock suspension of the acid precipitated toxin suitable for the repeated determination of potency and reconstitution of the solution for mouse assay is described. As much as 50-fold variation in the LD_{50} in mice was obtained by variation of the chemical nature of the diluent. A low titer was obtained when a solution of NaCl or phosphate, acetate or citrate buffer was used as the diluent; a higher titer resulted when 0.2% gelatin was incorporated in the diluent and still higher when it was replaced by 5.0% gastric mucin. The assay of the toxin diluted with water was about the same as when diluted with blood serum or a solution of 0.2% gelatin in 1.0% phosphate, autoclaved, but the LD_{50} was somewhat higher when the gelatin phosphate diluent was not sterilized by autoclaving but rather by Seitz filtration. Explanations of some of the discrepancies are suggested.

594. Inducing effect of irradiated leucovorin and derivatives in lysogenic microorganisms. ERNEST BOREK AND JOYCE ROCKENBACH*