In practice the two reactions produced two different products.

From Ia a flavone m.p. 138–139° (III) (Found: C, 78.10; H, 5.61. Calcd. for $C_{19}H_{16}O_3$: C, 78.06; H, 5.52) from I a different flavone m.p. 222–223.5° (IV) (Found: C, 78.15; H, 5.29. Calcd. for $C_{19}H_{16}O_3$: C, 78.06; H, 5.52) were isolated.

These results must be ascribed to the formation of two different intermediate β-diketones. As the Baker-Venkataraman rearrangement product was yellow, it might be formulated, according to an observation of OLLIS and WEIGHT[5], as ω-benzoyl-2-hydroxy-3-propionyl-acetophenone (II). Consequently it may be inferred that III is 6-methyl-8-propionyl-flavone and IV the corresponding isomer, 3,6-dimethyl-8-acetyl-flavone. These assignments were proved by alkaline hydrolysis of III and IV, which gave 3-propionyl and 3-acetyl-5-methyl-salicylic acids respectively, as well as aceto and propiophenone, characterized as 2,4-dinitrophenyl-hydrazones. The structures of the two salicylic acid derivatives were confirmed by comparing them with authentic samples. The formation of two different flavones therefore, depends on the experimental conditions, i.e. on the different temperatures. At 90°, in the Baker-Venkataraman rearrangement, the migrating benzoyl group is directed to the α-carbon atom of the acetyl chain; at 180–190°, in the Kostanecki-Robinson acylation, the same group is directed to the α-carbon atom of the propionyl chain.

Riassunto. Si riferisce sul diverso comportamento del 2-propionil-4-metil-6-acetilfenolo nella acilazione secondo Kostanecki-Robinson e nella trasposizione di Baker-Venkataraman.

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Breathing Fluid

Foetuses of mammals, including human foetuses, 'breathe' amniotic fluid[1,2]. Newborn mammals may survive complete submersion for considerable periods of time, dependent upon their stage of development. Signs of life have been observed in puppies up to 54 min after submersion in water[3]. Young rats have been reported to continue making respiratory movements for more than 40 min when, shortly after birth, they were submerged in water at 37°C[4]. This tolerance to asphyxia of the newborn, however, diminishes rapidly with age.

It has now been found that adult mammals submerged in a salt solution may breathe fluid for more than 2 h provided they obtain enough oxygen.

Experiments were done on adult white mice. In controls, all respiratory movements ceased approximately 1 min after submersion in saline[5] at 25°C. Animals drowned in 600 ml of saline containing 0.1% of hydrogen peroxide lived from 3 to 5 times as long. Unanesthetized mice submerged in 1500 ml of saline at 25° C which had been saturated with oxygen at 8 atmospheres pressure absolute (8 ata) in a specially constructed transparent tank, continued breathing fluid for periods lasting up to 40 min. Mice anesthetized with pentothal lived up to 2 h and 25 min after submersion in 1500 ml of saline which, after equilibration, initially contained approximately as much oxygen as ambient air at sea level[6].

These experiments clearly demonstrate the potential biological adaptability of adult mammals to a marine environment such as previously existed during ontogenesis and phylogenesis.

Zusammenfassung. Ausgewachsene weisse Laboratoriumsmäuse atmen untergetaucht in 600 ml einer isotonischen Salzlösung bei 25°C 3 bis 5 mal länger, wenn der Flüssigkeit 0,1% Wasserstoffperoxyd zugesetzt wird. In 1500 ml einer isotonischen Salzlösung, die bei einem Sauerstoffdruck von 8 atü (25°C) equilibriert wurde, können sie über 2 h lang Flüssigkeit «atmen».

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Free Amino Acid Pool in Strains of *Shigellae*

The existence of an internal amino acid pool in bacteria has been shown by several investigators[1–10]. MIZUNO et al.[4] first showed the presence of free amino acids within the cells of dysentery bacilli. In the studies on the metabolic activities of members of the genus *Shigella*[11], a number of amino acids were noted in the free amino acid pool of three strains of dysentery bacilli. The composition of the 'pool' of these strains grown in different media is reported in this communication.

The strains used were *Sh. flexneri 2a* (NCTC 8519), *Sh. flexneri 1a* (NCTC 8516), and *Sh. dysenteriae 6* (NCTC 6342) and were chosen because of their different nutritional characters[11]. The minimal medium in which the strain of *Sh. flexneri 2a* showed prompt growth was a chemically defined basal medium[12] supplemented with

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ammonium chloride (1%) as the sole nitrogen source; it was also able to grow with any of the 19 amino acids present singly[11,13]. The strain of *Sh. flexneri 1a* showed slow growth with ammonium chloride and was able to grow with several amino acids present singly in the medium, the exceptions being serine, proline, tyrosine, and isoleucine. Prompt and luxuriant growth was obtained with *DL*-glutamic acid, 0.01 *M*. The strain of *Sh. dysenteriae 6* was unable to grow in the basal medium with ammonium chloride or single amino acids as the nitrogen source and for its growth a mixture of three amino acids, *DL*-glutamic acid, *DL*-methionine and *DL*-tryptophan (each 0.01 *M*), was required.

The free amino acid pool was analysed after growth in the following media: (a) all three strains on 1.8% nutrient agar (New Zealand)[14]; (b) all three strains in the basal medium containing acid hydrolysed casein (1%) supplemented with *DL*-tryptophan 0.01 *M* and *L*-cystine, 0.0005 *M*; (c) strain 8519 in the basal medium containing ammonium chloride (1%); (d) strain 8516 in the basal medium containing *DL*-glutamic acid, 0.01 *M*; and (e) strain 6342 in the basal medium containing *DL*-glutamic acid, *DL*-methionine, *DL*-tryptophan (each 0.01 *M*) and *L*-cystine, 0.0005 *M*.

After overnight (about 18 h) aerobic growth at 37°C, the cells were harvested and washed once with cold double-distilled water and suspended in double-distilled water to contain between 20–30 mg dry weight of bacteria per ml. The free amino acid pool was present in the supernatant obtained after boiling the cell suspension at 100°C for 10 min[1], cooling the suspension and centrifuging at 3000 r.p.m. The supernatant was evaporated to dryness on a water bath and the residue was dissolved in double-distilled water using one-tenth of the volume of the original supernatant. The amino acids were detected by one-dimensional paper chromatography[4,9]. Known amino acids were used as markers to determine the positions of the fractions[15] and, in recording the results, following MANDELSTAM[9], the fractions were placed within quotations marks.

The composition of the free amino acid pool of the three strains of shigella grown in different media is shown in the Table. MIZUNO et al.[4] found amino acids spots other than glutamic, aspartic and alanine to be negligible in the strains of *Sh. flexneri* and *Sh. sonnei* examined by them. However, in the present study, a number of amino acids were noted in the internal amino acid pool of the shigella strains. The composition of the 'pool' varied with different media in which the cells were grown, but qualitative differences could not be noted in the composition of the 'pool' of the three nutritionally differing strains grown on the same medium. Only slight differences were seen in comparing the 'pool' of the strains grown on their respective minimal media with the 'pool' when grown in casein hydrolysate medium. For strain 8519, with the exception of proline, the 'pool' of the cells from both media was similar. Strain 6342, growing in the basal medium supplemented with four amino acids, showed an additional 'tyrosine' fraction. There were no qualitative differences in the 'pools' of strain 8516 grown in the basal medium supplemented with glutamic acid and grown in casein hydrolysate medium[16].

Composition of the free amino acid pool of *Shigellae* grown in different media (Cells were obtained after overnight growth at 37°C)

Medium of growth	Strain	Amino acid fractions					
		Leucine	Methionine	Tyrosine	Alanine	Glutamic	Aspartic
Nutrient agar	8519	+	+	+	+	+	+
	8516	+	+	+	+	+	+
	6342	+	+	+	+	+	+
Casein hydrolysate	8519	—	+	—	±	+	+
	8516	—	+	—	+	+	+
	6342	—	±	—	+	+	±
Ammonium chloride	8519	—	+	P	+	+	+
Glutamic acid	8516	—	+	—	+	+	±
GMTC	6342	—	+	+	+	+	+

+ Prominent spot in chromatogram; ± Faint spot in chromatogram; — Not seen in chromatogram; P Proline present; GMTC Medium containing glutamic acid, methionine, tryptophan and cystine.

Zusammenfassung. Die Zusammensetzung des «Satzes» (pool) freier Aminosäuren in drei in ihrem Stoffwechsel verschiedene Arten von Shigella, *Sh. flexneri 2a*, *Sh. flexneri 1a* und *Sh. dysenteriae 6*, die in verschiedenen Medien wuchsen, wurde untersucht. Während MIZUNO et al.[4] den Satz von Aminosäuren ausser Glutaminsäure, Asparagin und Alanin in den von ihnen untersuchten Shigellaarten für unwichtig hielten, wurde in unserer Untersuchung in dem Ansatz freier Aminosäuren der Shigellaarten eine Anzahl von anderen Aminosäuren charakteristisch gefunden. Die Zusammensetzung des «Aminosäuresatzes» war bei den verschiedenen Medien, in denen die Zellen wuchsen, unterschiedlich; doch konnten qualitative Unterschiede bei den drei Spezies, die im selben Medium wuchsen, nicht festgestellt werden.

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Department of Bacteriology, University College, Ibadan (Nigeria, West Africa), October 16, 1961.

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Translocation of Labeled Photosynthate from the Bloom Node Leaf to the Fruit of *Pisum sativum*[1]

Introduction. The specificity of translocation in *Pisum sativum* of phosphorus-32 from the leaf, in whose axil the pod is borne, to that pod has been established. Up to 90% of the phosphorus-32 translocated from the leaf to which the phosphorus-32 is initially applied was found in the pod at the same node[2]. The principal objective of the present study was to learn whether the same specificity exists for carbon compounds as exists for phosphorus.

Materials and Methods. Plants of *Pisum sativum* L. var. Alaska were grown in a controlled environment room having a 75°F light period (12 h) followed by a 65°F

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