after concentration, the peptide derivative is washed with water and recrystallized. Dicarbobenzoxy-L-lysylglycine ethyl ester,[7] (92% yield), $[\alpha]^{25}D$ — 12.1° (*c*, 5; ethanol),[4] m.p. 90–91°, phthalylglycycl-DL-alanyl-DL-phenylalanine ethyl ester (71% yield), m.p. 189–192°, (*Anal.* Calcd. for $C_{24}H_{25}N_3O_6$: N, 9.31. Found: N, 9.56, 9.30) and carbobenzoxy, glycyl-DL-phenylalanine ethyl ester,[2] m.p. 89–90°, were prepared.

Thus by the use of one reagent units can be added to either a carboxy or amino group of a peptide derivative.

(7) M. Bergmann, *et al.*, *Z. physiol. Chem.*, **224**, 26 (1934).

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RECEIVED DECEMBER 8, 1950

THE NECESSITY FOR ACTIVATORS IN THE BORON TRIFLUORIDE CATALYZED ALKYLATION OF BENZENE BY S-BUTYL METHYL ETHER

Sir:

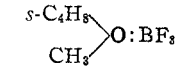
Attention has been directed recently to the necessity for the presence of traces of water or other activators in reactions catalyzed by Friedel–Crafts catalysts of the halide type. In the polymerization of ethylene,[1] isobutylene[2] and of diisobutylene[3] by boron trifluoride, traces of water are required. No suspicion of such necessity has existed where compounds known to coördinate boron trifluoride were involved.

We have found such a situation in the boron trifluoride catalyzed alkylation of benzene by *s*-butyl methyl ether. O'Connor and Sowa[4] reported that di-isopropyl ether reacts vigorously at room temperatures.

If a solution of *s*-butyl methyl ether and boron trifluoride in benzene (mole proportions 1:1:7) is prepared in apparatus exposed to the atmosphere, reaction (as noted by separation into two layers) occurs within a few days. If apparatus and materials are dried and atmospheric moisture excluded, no reaction occurs within three weeks.

Addition of water to this relatively anhydrous reaction mixture results in reaction. The added material forms a lower layer from which *schlieren* can be seen to rise until turbidity followed by large increase in the lower layer supervenes. Water reaches maximum effectiveness as an activator at a quantity equal to about 1 mole % of the boron trifluoride. Sulfuric acid and chlorosulfonic acid in similar quantities form lower layers and are even more effective than water. Methanol and acetic acid form no separate phase and are ineffective but ethanesulfonic acid, though it forms no separate phase, serves as an activator. No toluene is found in the reaction mixture; only the mono- and poly-*s*-butylbenzenes.

Boron trifluoride undoubtedly coördinates with the ether to



This complex, of itself, must be deemed relatively stable at room temperatures and not, as has been assumed, to decompose to a carbonium ion.[5] The additional intervention of a strong proton acid such as boron trifluoride monohydrate must be necessary.

In view of this behavior of secondary ethers, it is now questionable whether alkylation by analogous alcohols and esters can proceed by the process usually accepted[5]

$$RHO{:}BF_3 \longrightarrow R^+ + HOBF_3^- \qquad (1)$$

Alkylation by olefins must be similarly suspect.

(5) C. C. Price and J. M. Ciskowski, *ibid.*, **60**, 2499 (1938).

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RECEIVED DECEMBER 11, 1950

GROWTH RETARDING EFFECT OF SUBSTITUTED MALONONITRILES ON TRANSPLANT TUMORS IN MICE

Sir:

The report of Hyden and Hartelius[1] that administration of malononitrile specifically produced considerable increase of polynucleotides and of protein in the nerve cells, both in experimental animals and in human beings, led the authors to undertake a program of synthesis and testing of the effect of substituted malononitriles on transplant tumors in mice. It was hoped that substitution at the active carbon of the malononitrile would lead to a more widespread effect. This indeed was found to be the case and a preliminary report of the work was presented last April at the meeting of the American Association for Cancer Research.[2]

Nearly fifty substituted malononitriles have been prepared in our laboratory (some of them not yet recorded in the literature) which according to their structure were classified into four groups as follows: (A) R-CH-$(CH(CN)_2)_2$, (B) R-CH=$C(CN)_2$, (C) R_2-C=$C(CN)_2$, (D) R-N=N-$CH(CN)_2$. The compounds were tested in strain A, C3H and C57 mice bearing the following tumors: LCS-A, S-37, C3H-S, 6C3H-ED, Eo771 and myeloid leukemia C1498, respectively. Compounds of groups A and D produced no effect in which respect they were similar to the parent compound malononitrile and to sodium cyanide, which was also tested on the tumors. Twenty compounds of group B that were screened showed an effect of varying degree from no activity to 70% retardation of growth.

To this date the two most potent compounds were the *p*-nitrobenzalmalononitrile and the 5-nitrofuranalmalononitrile. These compounds were found to be very toxic, but 200 γ and 100 γ of each in 0.1 ml. of sesame oil in daily intraperitoneal injections were tolerated by the animals (average weight 25 g.). Treatment was initiated seven days after transplantation of the tumors and

(1) F. Hofmann, *Chem. Ztg.*, **57**, 5 (1933).
(2) A. G. Evans, G. W. Meadows and M. Polanyi, *Nature*, **158**, 94 (1946); Evans and Meadows, *J. Polymer Sci.*, **4**, 359 (1949).
(3) A. G. Evans and M. A. Weinberger, *Nature*, **159**, 437 (1947).
(4) M. J. O'Connor and F. J. Sowa, THIS JOURNAL, **60**, 125 (1938).

(1) Hyden and Hartelius, *Acta Psychiatrica et Neurologica*, Suppl. XLVIII (1948).
(2) Greenberg, Irish and Gal, *Cancer Res.* (Scientific Proceedings), **10**, 221 (1950).

the experiments lasted about fourteen days. The average number of animals was fifteen to twenty in each group. It was found that the *p*-nitrobenzalmalononitrile produced a retardation of growth of about 50% of the tumors C3H-S, and Eo771 and about 40% of S-37, while the 5-nitrofuranalmalononitrile showed 70% retardation of growth of C3H-S.

This work is being continued and the compounds that are found active are being followed up as to their fate and mechanism of action both *in vivo* and *in vitro*. Detailed results of this work will be reported elsewhere.

DIVISION OF BIOCHEMISTRY EMERY M. GAL
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RECEIVED NOVEMBER 20, 1950

STIMULATION BY THE ADENYLIC ACID SYSTEM OF AMINO ACID INCORPORATION INTO PROTEIN OF LIVER GRANULES

Sirs:

In view of the endergonic nature of peptide bond synthesis, and the key role of the adenosine phosphates in the energy transfer in biological oxidations, the adenylic acid system would be expected to participate at some stage in the over-all process of amino acid incorporation into protein. Up to the present the only evidence to support this expectation is: (1) Oxygen was found necessary for glycine uptake, while azide was inhibitory.[1] (2) The inhibitory effect of dinitrophenol on the incorporation of alanine into liver slice protein has been attributed to an interference with the synthesis of energy-rich phosphate bonds.[2]

On the other hand, very substantial evidence has been obtained for the participation of the adenylic acid system in the synthesis of peptide models: *p*-aminohippuric acid,[3] glutathione[4] and the acetylation of sulfanilamide.[5]

We have found that a liver granule fraction, nearly freed of soluble factors by draining, has relatively low activity with respect to amino acid incorporation. This system can be activated by the addition of magnesium ion, adenosine triphosphate, citrate and a mixture of L-amino acids. The activation occurred aerobically and not anaerobically.

A 1:5 homogenate of rat liver with ice-cold 0.9% KCl–0.4% $KHCO_3$ (saturated with 95% oxygen –5% CO_2 gas) was centrifuged for five minutes at 2,000 r.p.m. (680 × *g*) (International Refrigerated Centrifuge PR 1). The supernatant was centrifuged for thirty minutes at 4,000 r.p.m. (2700 × *g*). The supernatant liquid was decanted from the sedimented particles, and the inner walls of the centrifuge tubes blotted with filter paper. The particles were then suspended in an equal volume of KCl–$KHCO_3$ solution; 0.30-ml. aliquots of this preparation (containing 10 to 12 mg. of protein) were incubated with 0.03 ml. of each of the C^{14}-labeled amino acids in tubes containing substances dried *in vacuo* from aqueous solutions. The techniques of incubation and separation of radioactive protein have been described.[6]

Representative data for serine, glycine, leucine and phenylalanine are given in the accompanying table.

ACTIVATION OF RADIO-AMINO ACID UPTAKE BY ADENYLIC ACID SYSTEM

Activators	Micrograms[a] Labeled Carbon Per Gram Protein				
	Serine-β-C^{14} 0.9 mM.	Phenylalanine-β-C^{14} 0.26 mM.	Leucine-α-C^{14} 2.4 mM.	Glycine-α-C^{14} 1.2 mM. Initial	Glycine-α-C^{14} 1.2 mM. After SH[b]
None	0.30	0.10	0.40	0.34	0.30
	0.28	0.10	0.46	0.30	0.20
3 mM. ATP	0.33	0.10	0.43	0.33	0.20
	0.33	0.11	0.37	0.30	0.24
3 mM. ATP + 10 mM. $MgCl_2$	0.40	0.19	0.49	0.73	0.41
	0.41	0.20	0.59	0.76	0.43
3 mM. ATP + 10 mM. $MgCl_2$ + 6 mM. Citrate	0.75	0.37	0.99	2.48	0.82
	0.78		0.93	2.33	0.82
3 mM. ATP + 10 mM. $MgCl_2$ + 10 mM. MAGAPA[c]	0.56	0.42	1.08	3.69	1.32
	0.53		1.08	3.40	1.20
3 mM. ATP + 10 mM. $MgCl_2$ + 6 mM. Citrate + 10 mM. MAGAPA	1.50	0.80	1.79	5.24	1.86
	1.49(19)[d]	0.78(47)[d]	1.76(6)[d]	4.90(34)[d]	1.77(12)[d]
Above, omitting ATP	0.16	0.13	0.49	0.27	0.22
	0.16		0.56		

[a] Micrograms C*/g. protein = $\dfrac{\text{counts/min./g. protein}}{\dfrac{\text{M.w. of amino acid}}{12} \times \dfrac{\text{Specific activity of amino acid}}{1000}}$ [b] Mercaptoethanol treatment. [c] MAGAPA = 1.6 mM. L-methionine, 2.0 mM. L-aspartic acid, 3.0 mM. L-glutamic acid, 1.2 mM. L-arginine, 1.0 mM. L-proline and 2.1 mM. L-alanine. [d] Counts/min./mg.

With homogenates but not with tissue slices the radioactive protein obtained with radio-glycine

(1) T. Winnick, F. Friedberg and D. M. Greenberg, *J. Biol. Chem.*, **175**, 117 (1948).

(2) I. D. Frantz, P. C. Zamecnik, J. W. Reese and M. L. Stephenson, *ibid.*, **174**, 773 (1948).

(3) P. P. Cohen and R. W. McGilvery, *ibid.*, **169**, 119 (1947); **171**, 121 (1947).

(4) R. B. Johnston and K. Bloch, *ibid.*, **179**, 493 (1949).

(5) F. Lipmann, *ibid.*, **160**, 173 (1945).

(6) T. Winnick, E. A. Peterson and D. M. Greenberg, *Arch. Biochem.*, **21**, 235 (1949).