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INHIBITION OF OXIDATIVE *N*-DEMETHYLATION IN MAN BY LOW DOSES OF INHALED CARBON DISULPHIDE*

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Abstract—Healthy adult male volunteers were exposed for 6 hr to graded CS_2 concentrations between 10 and 80 ppm. It was found that oxidative demethylation of amidopyrine given orally in therapeutic doses was inhibited, as measured by urinary 4-aminoantipyrine excretion. The conversion (excretion) deficit was compensated for almost completely during the subsequent excretion phase. The inhibition in amidopyrine metabolism is reversible. Both the magnitude and duration of the inhibitory effect, as well as onset of maximal inhibition and the tendency for compensation of the deficit, increase with rising CS_2 doses. The inhibitory effect is no longer detectable at 18 hr after exposure to 20 ppm. Exposure to 20 ppm for 6 hr on five successive days produces an inhibitory reaction identical to that seen after single 6-hr inhalation of 40 ppm. The observed interfering effect which is due to inhibition of microsomal hepatic enzymes constitutes so far the most sensitive criterion of a selective CS_2-induced organ effect.

OXIDATIVE drug metabolism by hepatic microsomal mixed-function oxidases is inhibited in rats by inhaled carbon disulphide.[1] A significant reduction in the hexobarbital sleeping time is observed after 8-hr-inhalation of CS_2 concentrations as low as 20 ppm, which is the current threshold limit value in industry. The inhibitory action was demonstrated using aromatic and aliphatic hydroxylation and dealkylation processes. The effect is entirely reversible, its magnitude being closely related to the concentration of CS_2 inhaled.[2] The inhibition is intimately linked with the presence of CS_2 in the tissues.[3] There is essentially no disturbance of conventional liver function tests even when drug oxidation is maximally inhibited after 400 ppm CS_2/8 hr, which was the highest dose administered.[1,4]

These observations prompted an investigation as to whether the inhibitory effect also occurs in human subjects after low doses of CS_2. For this purpose we determined the contribution of hepatic mixed-function oxidases to the degradation of a therapeutic dose of amidopyrine to 4-amino-antipyrine (4-AAP). The excretion of the *N*-demethylation product before and after CS_2 inhalation should be taken as representative of alterations in microsomal enzyme activity.

Such investigation should afford a better means of assessing the maximum acceptable concentration of CS_2 in industry. This value is set in some countries at 20 ppm. The available literature does not include any data on the organ effects of such low CS_2 concentrations after acute exposures of humans. In addition, the establishment of a highly sensitive criterion of CS_2 action should also provide a basis for elaborating an exposure test for subjects working in CS_2-contaminated atmospheres.

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METHODS

Volunteers. Our experiments were conducted on 19 healthy male volunteers (aged 21–40 yr; body wt 65–80 kg). Nearly all persons were subjected to one exposure each at the individual CS_2 concentrations; they thus participated in several tests at intervals of at least 30 days. As has been shown previously,[1] this period is far in excess of the recovery period with regard to the enzyme inhibition which is completely reversible within 2 days. A few weeks prior to the commencement of the tests all volunteers were instructed to discontinue drug intake and to restrict ingestion of alcoholic beverages to moderate amounts.

Experiments. Groups of four persons each were exposed to different concentrations of CS_2 for 6 hr (invariably from 8.00 a.m. to 2.00 p.m.). When starting the experiment, the fasted individuals were given an oral dose of amidopyrine (7 mg/kg) in a gelatine capsule. After 2 and 3 hr they were given standard meals of two and one sandwiches, respectively. During the exposure period they were allowed to drink 1·25 l. (apple juice and mineral water). After the exposure, individual eating and drinking habits were resumed. Urine was collected at 3, 6, 9, 12, 16, 24 and 33 hr following the beginning of the exposure and amidopyrine administration. A blank urine sample was collected immediately before the assay. Each volunteer served as his own control. All control tests were performed under the conditions described above, except that CS_2 was added to the exposure atmosphere.

Exposure apparatus. A cubical airtight enclosure of approx. 8 m^3 with three glass walls and three tiled walls served as the exposure chamber. Depending on the desired CS_2 concentration, the continuous dynamic flow of the CS_2/air mixture drawn through the chamber could be adjusted to give 8–15-fold changes of air/hr. The CS_2/air mixture entered under uniform pressure through a vent at one edge. The exposure mixture was prepared in a spherical glass mixing vessel (14 cm dia.) by evaporation of liquid carbon disulphide (reagent grade, Merck/Darmstadt) into a rotametrically metered stream of air. Continuous dropwise addition of carbon disulphide according to the desired concentration was obtained with an automatic infusion apparatus (Perfusor type 71100, Messrs. Braun/Melsungen). Constant evaporation was maintained by heating the spherical mixing vessel over a 50° water bath. The carbon disulphide/air mixture was diluted to the desired concentration with ambient air in another larger mixing drum. Permanent circulation of the chamber atmosphere was achieved by a vent in the middle of the roof. The CS_2 concentrations actually prevailing within the chamber were monitored before and during the entire exposure period with an automatically recording i.r. analyzer (type Uras 1, Messrs. Hartmann & Braun AG/Frankfurt-M.) which was mounted outside and connected with the exposure chamber by a glass tube. This instrument for selective CS_2 analysis had been pre-calibrated by conventional colorimetric determinations of CS_2.

Determination of CS_2. Carbon disulphide in the exposure atmosphere was determined by the impinger method of Viles[5] and McKee[6] using the modification of Massmann and Strenge[7] in which the reactant diethylamine is replaced by piperidine, as suggested by Demann and Adelsberger;[8] the sensitivity of the method was thereby considerably enhanced.

Determination of 4-aminoantipyrine and acetyl-4-aminoantipyrine. 4-Aminoantipyrine (4-AAP) and acetyl-4-aminoantipyrine (N-AcAAP) were assayed in the urine by the method of Brodie and Axelbrod,[9] as modified by Schüppel.[10]

Statistical evaluation. The results obtained were statistically evaluated using Student's *t*-test.

RESULTS

Figure 1 represents normal excretion rates of the metabolites of amidopyrine, 4-AAP and N-AcAAP after a single oral dose of 7 mg/kg. From this pattern we will study the effect of inhalation of different CS_2 concentrations. In Figs. 2 and 3, this will be shown by plotting the means of the individual changes as per cent of the control values against time. Indeed, this type of diagram better reveals the change in the excretion profiles from deficit to excess after cessation of CS_2 exposure.

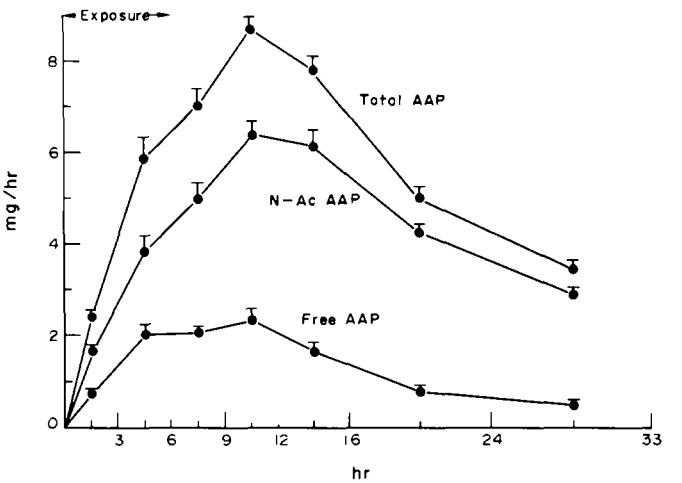

FIG. 1. Urinary excretion of amidopyrine (AP) metabolites after intake of 7 mg/kg AP. Normal values (mean ± S.E.) of 19 volunteers under simulated exposure conditions, but without CS_2.

1. *Single 6-hr exposure.* Figure 2 summarizes the results. Concentrations as low as 10 ppm CS_2 are sufficient to bring about a marked deficit in the formation (excretion) of 4-AAP which is statistically significant for the free (upper part) and total (lower part) 4-AAP, whilst with the acetylated form (middle) there is only a slight depression at this low concentration. Both the intensity and duration of the effect are enhanced with increasing CS_2 concentrations, the figures representing a well defined dose–response relationship. The time of maximal depression as measured by the excreted total 4-AAP shifts from 6 hr after 10 ppm to 12 hr after 80 ppm CS_2, whereas the amount of maximal deficit ranges from 14 to nearly 50 per cent. The period of inhibition (deficit of metabolite excretion) terminates between 14 hr (10 ppm CS_2) and 26 hr (80 ppm). Thereafter an overshoot occurs which is likewise dose-dependent. This surplus against the control values is not an actual excess in terms of an increase in the production (and excretion) of amidopyrine metabolites, but only compensates the loss incurred during the inhibitory period. A moderate overall enhancement of the formation of amidopyrine metabolites is observed but is statistically insignificant only after 40 and 80 ppm CS_2, as is shown in Fig. 4. However, the intersection of the "normal" excretion curves with the abscissa is clearly indicative of the limited

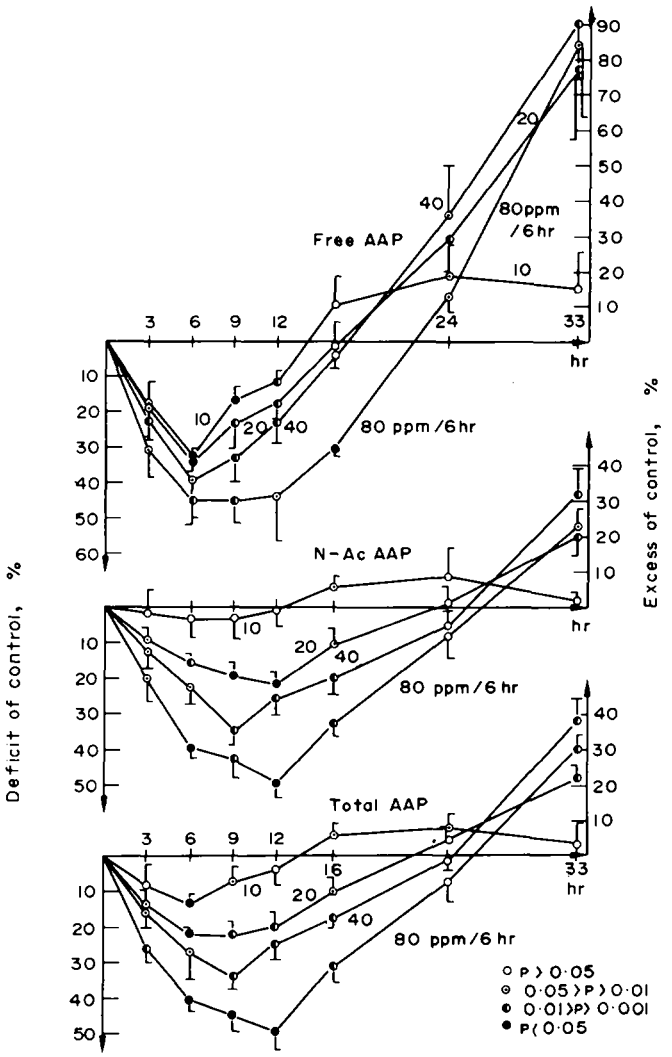

FIG. 2. Amidopyrine metabolites after oral intake of 7 mg/kg as compared to individual control values, during and after 6-hr-exposure to different concentrations of carbon disulphide. Results are expressed as means ± S.E.

duration of the inhibitory period. It should be borne in mind that the CS_2 exposure was continued up to the 6-hr mark on the time scale in Fig. 2. This would mean that in the 10 and 20 ppm series the degree of inhibition increases only up to the end of the 3-hr exposure period, or for another 6 hr in the 40 and 80 ppm series. Thereafter it falls towards the point of transition to the "excess" phase.

A further comparison of the upper and centre part of Fig. 2 reveals a significant difference in the "crossing over" from free to acetylated 4-AAP. With the exception of the 10 ppm series, the excretion of the N-acetyl product is subnormal for 25 hr on the average, whilst with free 4-AAP this period is nearly 8 hr shorter in duration.

2. *Duration of inhibition after single 6-hr exposure to 20 ppm* CS_2. The purpose of this experiment carried out on eight individuals was to investigate whether the

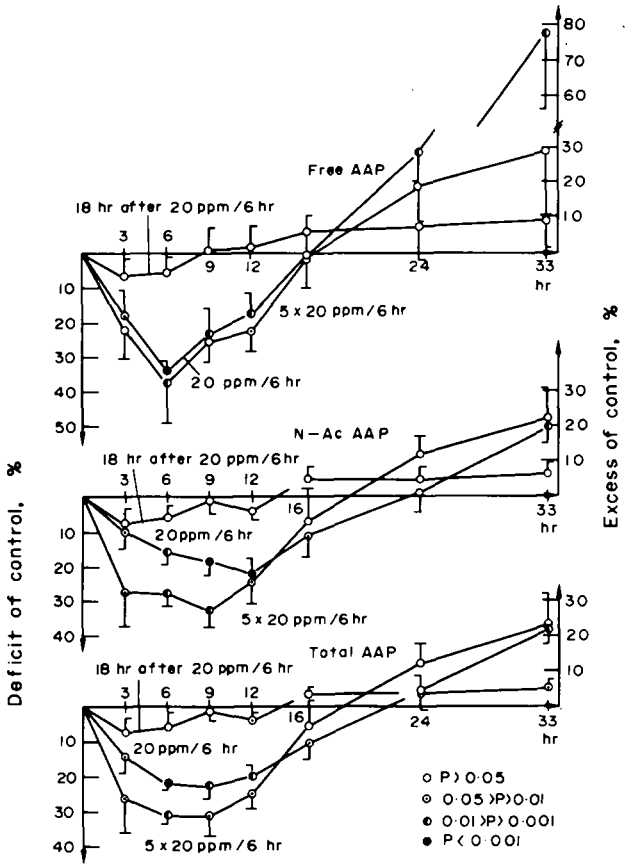

FIG. 3. Amidopyrine metabolites after 7 mg/kg p.o. as compared to individual control values. (a) Single 6-hr-exposure, amidopyrine intake 18 hr thereafter; (b) five 6-hr-exposures on consecutive days; (c) single 20 ppm CS_2 exposure 6 hr (same test as in Fig. 2, 20 ppm). Results are expressed as means ± S.E.

observed inhibition was still detectable at 18 hr after the 20 ppm exposure. The course of the curves in Fig. 3 clearly indicates that the amidopyrine ingested 18 hr following CS_2 exposure is metabolized at an approximately normal rate; there is only a slight and insignificant deviation from the controls. Inhibition by CS_2 thus is of only limited duration and disappears after 18 hr at the most.

3. *Repeated 6-hr exposures to 20 ppm CS_2.* A repeated exposure test was performed to elucidate whether cumulative inhibition might occur. Three volunteers spent 6 hr daily in an atmosphere of 20 ppm CS_2 on 5 successive days. Amidopyrine was administered at the beginning of the last exposure. It was seen (Fig. 3) that the excretion of total 4-AAP was reduced to a greater extent than during single exposure at the same CS_2 concentration. The overall excretion of 4-AAP and N-AcAAP (Fig. 4) was reduced by a slight but insignificant amount.

DISCUSSION

In the reported investigations it has been possible for the first time to demonstrate and quantitatively determine changes in organ function in man following controlled

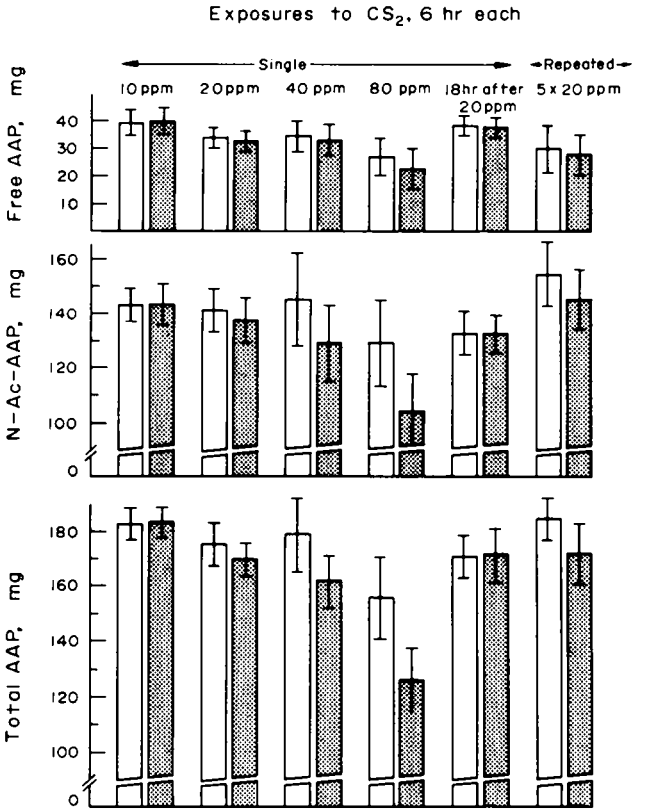

FIG. 4. Overall urinary excretion of amidopyrine metabolites during 33 hr following ingestion of 7 mg/kg. Results are expressed as means ± S.E.

exposure to very low CS_2 concentrations around and also below the maximum acceptable concentration (or threshold limit value). The mixed-function oxidases have been shown to be inhibited after only a single 6-hr exposure to 10 ppm of carbon disulphide. Repeated exposure resulted in only a slight increase of this effect which was barely significant. Previous investigations involving controlled CS_2 inhalation in man at far higher concentrations[11,12] had provided no indication of functional changes or clinical symptoms. Measurement of the impairment of mixed-function oxidases thus constitutes by far the most sensitive criterion of CS_2 action.

The reported findings in man are in confirmation of extensive experiments on the inhibition of drug metabolizing reactions in rats. It had been shown in these animal experiments that the oxidative degradation of amidopyrine, hexobarbital, antipyrine, acetanilide, acetophenetidine and trichloroethylene was distinctly inhibited at CS_2 concentrations as low as 20 ppm.[1,2] Because of the varying testing methodology applied it is not possible to make a direct quantitative comparison between these animal experiments and the results reported in this paper on human individuals; it would appear, however, that human beings are by no means less sensitive than the rat. Identity has been found especially with respect to the duration of the inhibition.[1,2]

Exposure to 10 ppm CS_2 over 6 hr has been sufficient to produce a statistically significant restriction in free 4-AAP excretion. The precision of the deviation determined in such a relatively small number of volunteers is ensured by the possibility of accurately standardizing the experimental conditions in the test, particularly with respect to physical exercise, food and fluid intake during and subsequent to the experiment. It is clear that such a high degree of precision cannot possibly be attained under field conditions; it can be expected, however, when considering the dose–effect relationship, that inhalation of 20 ppm CS_2 for 6 and 8 hr, respectively, will also result in a significant deviation of the amidopyrine metabolism, even under non-standardized conditions.

The enzyme inhibition is of short duration; it can be distinctly measured for not longer than 6 hr after exposure to 20 ppm. This would appear to suggest that the observed inhibition is dependent on the presence of CS_2 in the metabolizing enzyme system. Initial support for this assumption has been provided by *in vitro* experiments on rat liver microsomes. These had revealed that the inhibition of amidopyrine demethylation is relieved after removal (washing) of CS_2.[13] In separate experiments on rats[3] it had been seen that the half life of the "free" CS_2 in blood, which can be easily removed from the blood and tissues in a current of helium, is not more than 35 min, that of the so-called "acid-soluble" fraction being roughly twice as much; the same relation obtains in liver tissue. From experiments in man[14,15] the half life of free CS_2 in blood can be calculated at 32 min, which thus is in good agreement with the values determined in the rat.

The mechanism of this inhibitory action has as yet not been elucidated. Inhibition of the prosthetic group of mixed-function oxidases—the haemoprotein P_{450}—is being discussed by some workers.[16] They had observed a decrease in the cytochrome P_{450} content in the rat liver, but the experimental conditions had not been quite realistic, involving oral administration of 1 ml/kg CS_2 in oil solution. Even after exposure to 387 ppm (6 hr daily on 5 days/week for a period of 6 weeks) the microsomal cytochrome P_{450} content was markedly reduced.[17] Follow-up studies performed in this laboratory on rats under exposure conditions corresponding to those in the *in vitro* inhibitory tests (20–400 ppm CS_2/8 hr) had shown that the P_{450} content in the microsomal fraction does not decrease significantly.[18]

A similar inhibition of amidopyrine degradation is observed after disulfiram pretreatment in rats[19] and in man after administration of 500 mg and 100 mg as daily dose.[20] The initial conclusion to be drawn from these concurrent findings is that CS_2 inhalation gives rise to the formation of CS_2 metabolites which have a similar structure as the dithiocarbamates and become active in enzymes due to chelation of catalytically active metals. However, the reverse route is also conceivable, involving the metabolic release of CS_2 from disulfiram (and dithiocarbamates) and the direct or indirect reaction of its metabolites with mixed-function oxidases. Elucidation of this question, however, is subject to further relevant investigations.

So far, inhibition of amidopyrine degradation by foreign compounds has been rarely described. A comparable inhibition was found to occur in rats after the action of ethanol,[21] however, in this inhibition a mechanism differing from that in our reaction should be operative.

The reported experiments are of practical significance in two respects: On the one hand we are led to assume that inhibition of the oxidative drug metabolism in CS_2-

exposed workers is associated with the intensified or prolonged action of various therapeutics. This, in turn, gives rise to the question whether and to what extent such intensification of drug action can be tolerated. When establishing a maximum acceptable concentration for CS_2 at the work site, this question should be considered. Such an inhibitory action should be allowed for only to a limited extent, owing to the fact that in a large variety of drugs the magnitude and frequency of side effects can be distinctly enhanced by even moderate degradative inhibition. It is clear that drugs taken frequently and virtually uncontrolled, such as analgesics (aminophenazone, phenacetin, phenylbutazone and others) and hypnotics (barbiturates, glutethimide, etc.), as well as oral antidiabetic drugs and anticonvulsants, should be considered in the first place when setting CS_2 exposure limits. Especially in the case of highly cumulative agents (e.g. phenylbutazone, hydantoin derivatives), an excessive and potentially critical elevation of the expected "normal" plasma and organ levels may be a significant factor.

Inhibition of drug metabolism might also prove of value as a sensitive exposure test in workers handling CS_2. As intimated above, it will not be possible under field conditions to attain the accuracy of experimental exposures. Considering the relatively short duration of the inhibitory action, such a test would invariably have to be conducted during or immediately after the CS_2 exposure. Determination of free 4-AAP could serve as a particularly sensitive criterion involving collection of the urine passed during the first 6 hr after taking the test drug; a 24-hr-urine sample would affect or even totally mask the actual deviation, due to the subsequent "overshoot".

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REFERENCES

1. K. J. FREUNDT and W. DREHER, *Naunyn-Schmiedebergs Arch. Pharmak.* **263,** 208 (1969).
2. K. J. FREUNDT and P. KUTTNER, *Naunyn-Schmiedebergs Arch. Pharmak.* **264,** 232 (1969).
3. K. J. FREUNDT and E. SCHNAPP, *Naunyn-Schmiedebergs Arch. Pharmak.* **266,** 324 (1970).
4. R. KÜRZINGER and K. J. FREUNDT, *Naunyn-Schmiedebergs Arch. Pharmak.* **264,** 261 (1969).
5. F. J. VILES, *J. industr. Hyg.* **22,** 188 (1940).
6. R. W. MCKEE, *J. industr. Hyg. Toxicol.* **23,** 484 (1941).
7. W. MASSMANN and K. STRENGE, *Arch. Toxikol.* **16,** 58 (1956–1957).
8. W. DEMANN and A. ADELSBERGER, *Glückauf* **75,** 556 (1939).
9. B. B. BRODIE and J. AXELROD, *J. Pharmac. exp. Ther.* **99,** 171 (1950).
10. R. SCHÜPPEL, Diss. Math.-nat. Fakult. Univ. Tübingen (1968).
11. B. SOUČEK and E. PAVELKOVÁ, *Pracov. lék.* **5,** 181 (1953).
12. J. TEISINGER and B. SOUČEK, *J. industr. Hyg. Toxicol.* **31,** 67 (1949).
13. W. KROMER and K. J. FREUNDT, *Naunyn-Schmiedebergs Arch. Pharmak.* **266,** 381 (1970).
14. R. W. MCKEE, C. KIPER, J. H. FOUNTAIN, A. M. RISKIN and P. DRINKER, *J. Am. med. Ass.* **122,** 217 (1943).
15. H. DEMUS, *Faserforsch. Textiltechn.* **4,** 22 (1953).
16. E. J. BOND and F. DE MATTEIS, *Biochem. Pharmac.* **18,** 2531 (1969).
17. J. A. SOKAL, *Biochem. Pharmac.* **22,** 129 (1973).
18. K. J. FREUNDT and K. SCHAUENBURG, *Naunyn-Schmiedebergs Arch. Pharmak.* Suppl. Vol. **270,** R 35 (1971).
19. T. HONJO and K. J. NETTER, *Biochem. Pharmac.* **18,** 2681 (1969).
20. K. J. FREUNDT and W. DIESNER, (to be published).
21. R. SCHÜPPEL, *Naunyn-Schmiedebergs Arch. Pharmak.* **265,** 156 (1969).