ness. This can lead to an unreal situation by implying that the possible scale is percentagewise 1–100, yet, actually, the possible range is more nearly 30–80 (from the effectiveness of a placebo to the maximum effectiveness of morphine). Use of the ratio of first and third doses, where the third falls on a line near the asymptote, thus ties the situation down to the possible.

Heretofore we have always worked with average pain. In the present study this has been broken down for the first time into 2 distinct degrees: (1) See curve A of the Fig. 1. Reasons have been presented why this represents, initially (first dose level), severe pain; (2) Curves B and C (not different from each other, but different from curve A) represent pain more completely relieved by the 1st dose of morphine. A system has been presented for indicating and following the intensity of pain in a group of individuals. This has been expressed as an index that permits mathematical validation of difference from one interval to another. Finally, a system has been described for comparing the effectiveness of 2 analgesic agents on really severe pain, agents which are undifferentiable when studied with average pain.

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Methods of Obtaining Quinones from Flour Beetles

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Flour beetles *Tribolium confusum* J. du V. and *Tribolium castaneum* (Herbst) (*1*) secrete from thoracic and abdominal glands derivatives of *p*-benzoquinone (*2*). Because these quinones occur naturally and since similar synthetic quinones have profound biological effects (*3*), investigations into the chemical and biological significance of the beetle secretion have been under way for a number of years. To obtain an ample supply of test substance we have improved existing methods (*4*) of culturing and extracting the insects.

Culturing. Eight ounces of finely ground Wheatsworth graham flour with 5% Fleischmann's pure dry yeast added were introduced with 2000 insects in a quart Mason jar. The jar was sealed by a soft rubber gasket, a screen wire circle (40 mesh), and the outer part of the screw cap. About 40 jars each were stacked in iron trays 38 in. long, 9 in. wide, and 3 in. deep. The cultures were kept in a conditioned dark room at 32° C and 80% RH. After 6 weeks, the contents of 4 jars each were sifted through a 22-mesh plastic cloth screen. The portion remaining on the sieve comprising beetles, larvae, pupae, shed skins, and other coarse material was transferred to a clean Mason jar that was sealed as before except that a half-circle of cardboard covering the lower half of the jar opening was used in place of the wire screen. The surface of the cardboard was glossy outside and dull inside the jar. These jars were laid on the beetle separator (Fig. 1) which consisted of a support, a polished steel funnel, and a beetle receiver. The insects climb up the cardboard and fall through the funnel into the receiver. An extremely clean batch of beetles is obtained in a few hours' time without further attention.

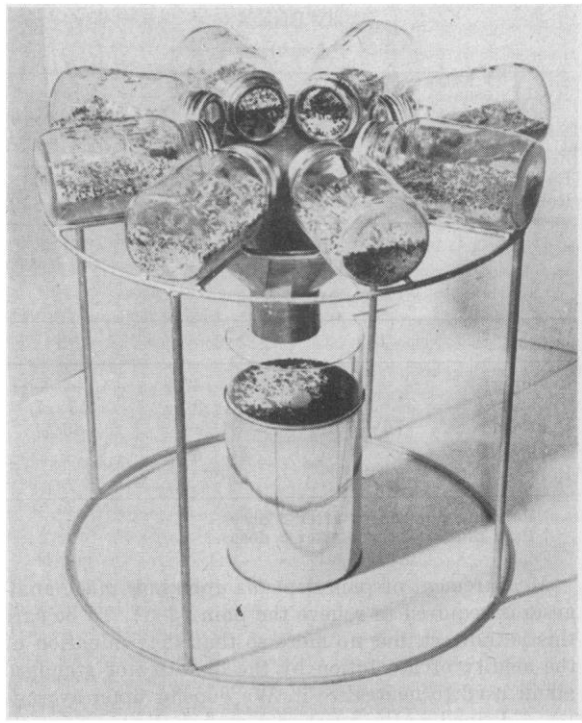

FIG. 1. Beetle separator.

In a widely used method of culturing, the jars are sealed by cloth sheeting and placed upright. We have found that our procedure yields a greater number of insects. From 10 conventional cultures each started with 2000 insects and maintained in the described environment for 6 weeks a little over 1000 additional beetles were obtained in the average per culture. With our present method, 10 jars produced in the average more than 3000 additional insects per culture. This is due, possibly, to better ventilation in the jars.

When beetles were collected for extraction, the receiver was exchanged for a cylindrical metal flask fitting the bottom of the funnel. The flask contained some dry ice and was kept surrounded by dry ice-alcohol in a Dewar flask. In this manner, the insects falling through the funnel were instantaneously anesthetized by the CO_2 atmosphere in the flask and killed by deep freezing. The quinones were thus pre-

[1] Thanks are due Louis M. Roth for his active interest in the development of the extraction method and for furnishing us with cultures of *T. confusum* and *T. castaneum*.

served since they solidified immediately at this low temperature.

Extraction of Quinones. These quinones are decomposed by light, heat, moisture, and some organic solvents. They may change into undefinable polymers or oily brown masses. Also, they are extremely reactive toward compounds occurring in living matter; they form, for instance, additional compounds with proteins including nucleoproteins, amino acids, enzymes, and vitamins (*5*).

Previous procedures of isolating the beetle secretion (*4*) are time consuming and yield very little of the unchanged quinones. We employ the mild conditions and efficient procedure of high-vacuum extraction (*6*). The apparatus consists of a 1-l flask, a series of 3 traps, and a high-vacuum pump. The flask is kept at 40° C by a water bath while the traps are thermostated to 0, −20, and −78° C, progressively. About 50,000 frozen insects (200 cc by volume) are placed in the flask. Upon evacuating, yellow quinones begin to deposit in the first trap. The main portion is collected in a few hours. After 24 hr there are practically no quinones left in the beetles, but a slight additional amount may be recovered by increasing the temperature of the flask to 95° C. Finally, the yellow compounds including some yellow oil are found in the first 2 traps, while the remaining volatile matter, mainly water, is found in the coldest trap. The crude quinones melt between 29 and 53° C. They may be purified by extracting with pentane, evaporating the solvent, and subliming under vacuum.

One insect weighs about 1.8 mg (*7*). Almost 60% of its weight consists of matter volatile under high vacuum and it produces about 25 gamma of crude quinones. We have observed also traces of reddish, greenish, and dark blue substances along with some sweet smelling colorless oil in the extractable portion of these beetles.

For studying the effects of these insects upon and in contact with flour, the volatile matter is adsorbed directly on flour. The traps are replaced by a 1-l flask with finely ground flour spread in a thin layer over its bottom and cooled by dry ice-alcohol. During extraction, the volatile matter deposits on the flour and the inner walls of the flask. After extraction, the flask is removed and lightly stoppered to allow for expansion of the gases while the flask is warming to room temperature. After several hours the extracted quinones complete their chemical reaction with the flour as indicated by the reddish color developing on the flour, if sufficient insects have been used in relation to the amount of flour. No visible color change is ordinarily detectable on 1 lb of bleached white flour at concentrations up to 10 mg of beetle secretion as obtained from about 400 insects.

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Products of the Reaction between Thiamine and Ingredients of the Plants of *Allium* Genus: Detection of Allithiamine and Its Homologs

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Fujiwara *et al.* (*1*) reported that when thiamine is heated at pH 8 with the alcoholic extract of the garlic (*Allium sativum* L. var. *japonicum* Kitamura), its thiochrome test becomes negative but it is restored by treating with cysteine, and that a crude product of this reaction, in spite of its negative thiochrome test, still possesses a remarkable activity similar to that of thiamine and is absorbed from the intestinal canal more rapidly than thiamine. They named the product allithiamine.

The present authors (*2*) have succeeded in isolating pure allithiamine and have established its structure as 2-(2′methyl-4′-aminopyrimidyl (5′))-methyl-formamino-5-hydroxy-Δ^2-pentenyl (*3*) allyldisulfide(I).

However, in this reaction, besides allithiamine, the formation of its homologs having other alkyl groups than allyl may be assumed, especially in the reaction of thiamine and other plants of *Allium* genus whose sulfur-containing ingredients have not been clarified. For confirmation of the formation of such homologs it is necessary to find a method which can detect them distinctly.

On the other hand, the study of the mechanism of the reduction of allithiamine(I) was reduced by cysteine(IV) into thiamine(V) and at the same time, the liberated allylmercapto group combined with cysteine to form S-allylmercapto-cysteine(VI), which had already been obtained

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