head. The sequence in which appendages received the maximum radiophosphorus content was the same regardless of the point of injection; the wings were reached by the radiophosphorus first after injection in the abdomen and last after injection in the head. When the insect was injected in the head the first pair of legs consistently showed a decrease after an initial rise to a maximum radiophosphorus content. Apparently the injected solution was very poorly mixed with the body fluid in so short a distance. Complete mixing of the body fluid required about 25 min. In *A. tristis* the order in which the radiophosphorus reached the appendages was the same, with a time to a maximum of about 35 min. Not enough experiments were performed with *T. molitor* to show the sequence in which the radiophosphorus reached the appendages. The time for uniform mixing can be estimated at 8–10 min.

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An Instance of the Occurrence of Carcinogenic Substances in Certain Barnacles[1]

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The chromatographic fractionation of some extracts obtained from a sample of the thatched barnacle (*Tetraclita squamosa rubescens*) showed on lime and alumina columns the presence of several zones that displayed intense blue fluorescence in ultraviolet light. Some of the fractions were crystallized and gave in hexane solution extinction curves that were typical for polycyclic aromatic hydrocarbons. The carbon and hydrogen content of one of these fractions (5 mg from 1 kg barnacles), as well as the observed molecular weight, corresponded to the values calculated for benzpyrenes. Furthermore, a spectroscopic examination, although also indicative of accompanying isomers or close analogs, demonstrated the presence of 3,4-benzpyrene in the crystalline mixture. Consequently, this particular sample was tested on mice for carcinogenic activity.

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The material was dissolved in tricaprylin, 5 mg/ml. Twelve male C_3H mice, 3 months old, received a single subcutaneous injection of 0.5 mg in 0.1 ml; 12 additional mice were injected with 0.25 mg in 0.05 ml. The mice were maintained on Purina dog chow and an unlimited supply of water. They were examined weekly for the presence of progressively growing tumor at the site of injection.

Four of 12 mice receiving 0.5 mg of the material developed subcutaneous tumors in 16, 17, 19, and 20 weeks following injection. Two of 12 mice receiving 0.25 mg developed tumors in 17 and 19 weeks. The remaining 18 mice were alive and free of tumor 36 weeks after injection.

The mice with tumors were sacrificed when the tumors reached 1–2 cm in diameter. On histologic examination, all 6 were seen to be spindle-cell sarcomas with local invasion of areolar and muscular tissue. Morphologically they were indistinguishable from tumors induced with 3,4-benzpyrene and other polycyclic carcinogenic hydrocarbons (*1*). The first tumor to be noted was transplanted into six C_3H mice and grew vigorously within 10 days, maintaining its sarcomatous appearance.

Previous data (*2, 3*) showed that 80–90% of C_3H male mice developed sarcomas within 20 weeks after the subcutaneous injection of 0.25–0.5 mg of 3,4-benzpyrene dissolved in tricaprylin. The incidence of approximately 25%, and the longer latent period of the tumors in this investigation, suggest that the material tested contained 10–40% of the active carcinogen, assuming that 3,4-benzpyrene was the only such compound present and that other substances in the sample did not alter the carcinogenic reaction. The presence of an active carcinogen was unquestionably demonstrated.

Comparative work on barnacles has shown that the polycyclic aromatic hydrocarbons do not constitute normal metabolic products but may reach these organisms accidentally. The possibility of tarry materials, from ships or submarine oil wells, being carried to the filter-feeding intertidal sedentary animals constitutes a potential external source for aromatic polycyclic hydrocarbons. We may also mention that the wooden pilings from which the material was collected at Corona del Mar, Calif., had been given a surface creosoting 10 years previously.

It has been observed that the goose barnacle (*Mitella polymerus*), collected from another habitat (on and among the mussels growing on the pier pilings at the Scripps Institution of Oceanography, La Jolla, Calif.), also yielded a fluorescent fraction whose extinction curve was indicative for the presence of 3,4-benzpyrene, although the quantity of this fraction was at least 10 times less than mentioned above. In contrast, our fluorescing fractions from *Tetraclita squamosa rubescens*, originating from rocks near La Jolla, were found to be of different nature and free of benzpyrene or similar compounds.

A detailed report on the isolation and characterization of polycyclic aromatic substances from barnacles will be given elsewhere.

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An Analog of Histamine that Stimulates Gastric Acid Secretion without other Actions of Histamine

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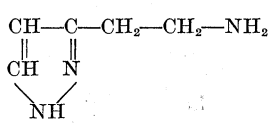
During a survey of the stimulatory and inhibitory actions of chemical analogs of histamine on gastric secretion, one compound was found that possessed the unique property of stimulating gastric acid secretion without producing any of the other pharmacologic actions of histamine. The compound is 3-(β ethylamine) pyrazole (compound XXIV). Its structural formula is:



It will be seen that this is an isomer of histamine in which the ring has a pyrazole instead of an imidazole configuration. The relationship of the side chain to the =N— is the same as in histamine. The compound was first synthesized and studied by Lee and Jones (*1*), who found that it did not contract the isolated guinea pig ileum and that it did not lower blood pressure in cats. In the anesthetized dog we have found compound XXIV to be about 1/700th as potent as histamine in depressing blood pressure.

Compound XXIV stimulates acid secretion in dogs with pouches of the entire stomach when the hydrochloride is administered subcutaneously, intramuscularly, intravenously, or orally. By the subcutaneous route the ED_{50} of the hydrochloride (dose required to produce 50% of the maximal secretory rate) is 8 mg/10 min in comparison with 0.12 mg/10 min for histamine diphosphate. The maximal secretory rate attainable with compound XXIV is the same as with histamine. The pepsin concentration of the juice secreted in response to compound XXIV is not significantly different from that stimulated by histamine.

The hydrochloride of compound XXIV has been injected into 20 human subjects by the subcutaneous and intramuscular routes in doses of 10–50 mg. In no instance have any of the characteristic side effects of histamine been observed, including triple response at site of injection, headache, or flush. The 50-mg dose produced a greater output of HCl than 0.01 mg/kg of histamine diphosphate. Doses of 100 mg produce side effects similar to those seen with histamine.

The existence of this histamine analog with specificity for site of action is in keeping with the known differences between the action of histamine on the gastric glands and its action at other sites. These include (*a*) failure of antihistaminic drugs to counteract action of histamine on gastric glands, and (*b*) inhibition by xanthine alkaloids of actions of histamine at other sites but potentiation of the gastric secretory action.

Compound XXIV may prove useful for routine clinical testing for achlorhydria, not only because it would eliminate the undesirable side reactions that follow histamine, but also because it would be possible to produce stronger stimulation of acid secretion and thus provide more clear-cut results in borderline cases. It may also prove useful for the investigation of the maximal secretory capacity of the human stomach in health and disease.

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Recovery of Tumor Cells from Effects of the Tumor-inducing Principle in Crown Gall

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When the tumor-inducing principle associated with the crown-gall bacterium acts on cells of plant species such as *Helianthus annuus* (*1*) and *Vinca rosea* (*2, 3*), an abrupt and irreversible change in the behavior of these cells occurs. Following the transformation process, proliferation of the altered host cells becomes an automatic process that is independent of the inciting bacteria. The cells of the resulting neoplasm are characterized by excessive powers of proliferation and limited powers of differentiation. Competence for organization appears to have been lost as a result of the action of the tumor-inducing principle. Tumor cells of this type are transplantable, and when bacteria-free fragments are implanted into a healthy host they develop into typical uncoordinated crown-gall tumors.

When, on the other hand, this same tumorogenic principle acts on cells located close to the top of a plant species such as *Kalanchoe daigremontiana,* the altered cells appear at first to be of an undifferentiated type. As the tumors grow, however, there results, in place of the characteristic neoplasm, an overgrowth composed not only of uncoordinated tumor cells but of cells that are organized into morphologically complex structures (*4*). The question as to whether these structures result from the growth of normal cells that have been stimulated to develop by the expanding tumor, or whether the morphologically abnormal shoots are composed of altered cells that have acquired a ca-