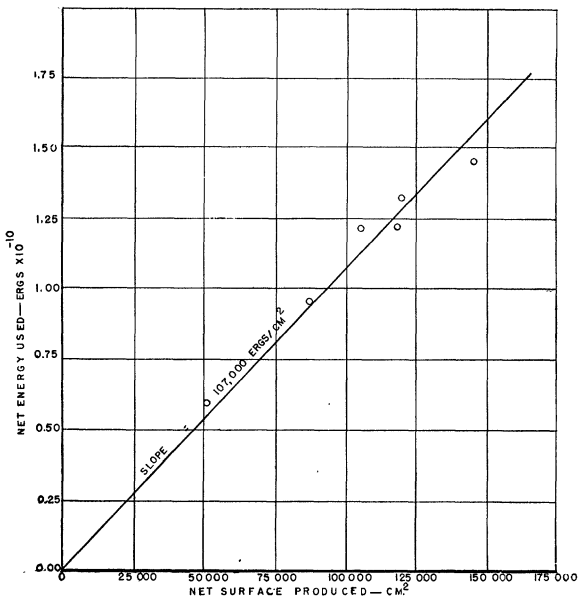

FIG. 1. Surface energy of quartz sand.

the chamber. Under these conditions, the kinetic energy input balanced the energy output as heat when the calorimeter was revolved for a short length of time. No breakage occurred under these conditions, and all of the energy input became heat energy through friction of the tumbling mass. However, when a brittle material such as quartz was added to the chamber and was comminuted, the energy output was about 10%–20% lower than the energy input. By the reasoning already outlined, this difference was considered to remain on the brittle material as surface energy.

The total surface on the brittle solid was measured both before comminution and after comminution by the rather well-known method of BET adsorption isotherms, using CO_2 gas as the adsorbate. The net surface produced during the comminution was thus obtained by subtraction and, divided into the net energy difference, gave the specific surface energy of the brittle crystalline solid.

The calorimeter was used to derive the surface energy of quartz by making a series of determinations on a glass sand made up of quartz grains. The net energy consumption—that is, the energy to create new surface—was plotted against the net surface produced (Fig. 1). A straight line is seen to average the six points determined and, moreover, to pass through the origin of the plot as it should. The slope of this line is considered to give the average total surface energy of the quartz sand. It is 107,000 ergs/cm². The deviations of the individual points from the straight line are not large. The order of magnitude of this surface energy figure for quartz is not seriously in conflict with the figures mentioned earlier in this article, and seems to agree in a qualitative way with the difference in hardness of a substance like quartz and a substance like mercury with a surface energy of approximately 473 ergs/cm² at room temperature.

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Some Derivatives of Diphenyldisulfide with Antispirochetal Activity[1]

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Among the few synthetic and natural disulfides (gliotoxin) which have been studied chemotherapeutically, no substances of therapeutic importance have been found. This may be the result of the low activity of these substances or of their unfavorable chemotherapeutic ratio. Research on bis(4-aminophenyl)disulfide makes it seem possible that the disulfides constitute a class of chemotherapeutic agents distinct from the sulfonamide drugs, and active through a different mechanism, which has not yet been defined (*4*).

For some time two of us (P. P. and L. R., *5*) have focused our attention on bis(2-aminophenyl)disulfide and its related compounds. McDonagh (*1, 2*) recognized the activity of bis(2-aminophenyl)disulfide against *Spirochaeta pallida*; we have since found that this substance is also active against *Borrelia recurrentis* (*3, 6*).

In view of the antispirochetal activity of bis(2-aminophenyl)disulfide, we thought it would be interesting to see whether correlations analogous to those existing between *p*-amino-substituted disulfides and sulfonamides in *coccus* infection would hold good also for spirochetal infection in the series of *o*-amino derivatives. For this purpose, a series of *o*-amino sulfamides was prepared, that is: 2-aminobenzenesulfonamide, 2-(2-aminobenzenesulfamido)-pyridine, 2-(2-aminobenzenesulfamido)-4-methylthiazole, and 2-(2-aminobenzenesulfamido)benzenesulfamide, in order to see if these substances would show antispirochetal activity greater than that already observed in bis(2-aminophenyl)disulfide.

Research on the activity of these compounds against *Spirochaeta hispanica* and *Treponema brucei* in mice was performed by Prof. D. Bovet at l'Institut Pasteur, Paris, and has given completely negative results (*6, 7*). This confirms the distinction between the class of disulfides and that of the sulfonamides as chemotherapeutic agents.

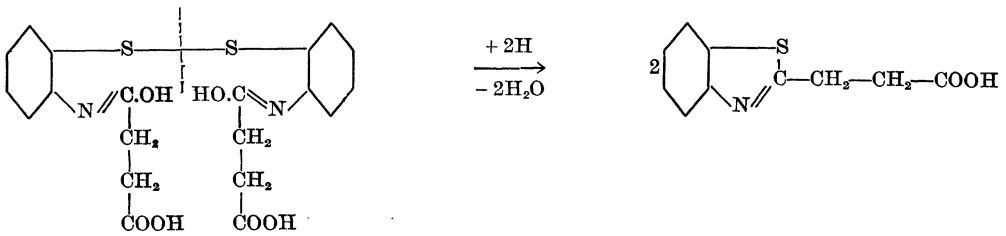
Among the derivatives of bis(2-aminophenyl)disulfide previously synthesized by us, one of the most interesting was the *N*-disuccinyl derivative (*3, 6 7*). Although its average lethal dose (13 mg per 20-g mouse) is only a

[1] Read at the First International Congress of Biochemistry (Section X), Cambridge, August 19–25, 1949.

little larger than that of bis(2-aminophenyl)disulfide (10 mg per 20-g mouse), the succinyl derivative is tolerated better at therapeutic doses and is clearly superior in its chemotherapeutic activity against *Borr. recurrentis*. In many experiments on *Borrelia*-infected mice, after a single injection of 4 mg of the succinyl derivative, about 50% were completely cured, and in the rest the infection took a far less serious course. This was deduced from the decreased number of organisms in the blood stream.

It is important to note that the molecular weight of the succinyl derivative is almost double (492) that of the free amine (248) and consequently, at the same weight, the molar concentration of the succinyl derivative is about half that of the amine. We must also note that the free amine is fat-soluble and the succinyl derivative is water-soluble; this difference influences, of course, the absorption and elimination of the two substances.

Bis(2-succinylaminophenyl)disulfide, when reduced by H_2S, cysteine, or ferrous hydroxide, in an acid, neutral, or alkaline medium does not give the corresponding thiol; instead it gives benzothiazolpropionic acid (BTP) (mp 108°–109° C; methyl ester 57°–59° C), which is immediately formed from the thiol by elimination of a molecule of water.



Since the same transformation might occur *in vivo*, it seemed possible that the activity of the compound was not due to the disulfide group, but to the benzothiazole derivative which arises from it.[2] This possibility could be investigated either by studying the action of the succinyl derivative and of benzothiazolpropionic acid on a certain number of microorganisms, or by examining the action of derivatives, substituted in such a way as to prevent their transformation into benzothiazoles. For a substance which possesses such a property we have chosen the bis(2-methylaminophenyl)disulfide, mp 72°–73° C, which was prepared in good yield from asymmetric methylphenylthiocarbamide.

The succinyl derivative and benzothiazolpropionic acid were tested biologically by Dr. Nascimbene and Prof. Babudieri. We also thank Sir C. Harington, the director of the National Institute for Medical Research, Hampstead, London, for arranging for Dr. Fuller to study the activity of these two substances against *Streptococcus hemolyticus, Staphylococcus aureus,* and *Escherichia coli.*

Results, briefly, are as follows: Bis(2-succinylaminophenyl)disulfide (Na salt), besides being considerably active against experimental infection in mice with *Borrelia recurrentis* (*3*), is also somewhat active against *Leptospira ictero-haemorrhagiae in vitro*[3] (growth is considerably inhibited at a concentration of 1: 12,000, completely at 1: 6,000). It has a definite action against *Staphylococcus aureus*[4] and *Streptococcus hemolyticus*,[5] less definite against *Escherichia coli*[6] and *Eberthella typhosa*[7] and has no action against a paratubercular strain[8] (Babudieri, Fuller). It has no action against *T. brucei* in mice (Babudieri). Benzothiazolpropionic acid (Na salt), under the same conditions, is completely inactive against all the organisms and infections mentioned (Nascimbene, Babudieri, Fuller). Bis(2-methylaminophenyl)-disulfide[9] is very active against *Staphylococcus aureus* and *Streptococcus hemolyticus* (complete bacteriostasis at concentrations of 1: 20,000 and 1: 100,000, respectively. It has a similar activity against *Leptospira ictero-haemorrhagiae* (complete bacteriostasis at a concentration of 1: 200,000). There is also a slight effect on *T. brucei* so that when ten mice were infected with *T. brucei* and treated for 2 days successively with 10 mg of the product, only two mice died after 48 hr, two after 3 days, and six after 4 days, while all the control mice died before 48 hr. This proves that the chemotherapeutic activity of these compounds is due to the molecule of the disulfide as such, which probably interferes with some essential metabolite by oxidizing or keeping oxidized a substance which should be reduced.

Among the enzymes which might be inhibited by disulfides, we first thought of sulfydryl enzymes. Such enzymes are inhibited by *p*-chloromercuribenzoate, certain trivalent organic arsenicals, and iodoacetamide. It is known that the pyruvate-oxidase system belongs to this group of enzymes. Prof. R. A. Peters, director of the Department of Biochemistry, Oxford, has demonstrated the inhibitory activity of bis(2-succinylaminophenyl)disulfide on the pyruvate-oxidase system in pigeon brain tissue. The concentration at which the system is inhibited (0.5–1.0 mg/3 ml) is about that of the chemotherapeutic activity on *Borrelia recurrentis*.

It is concluded that among the aromatic disulfides,

[2] There might be competitive inhibition with some essential metabolite. It is well known that benzimidazole, isostere with benzothiazole, inhibits the action of adenine (*8*).

[3] Strain Zaan, in Korthof's medium.

[4] Strain Oxford, in broth.

[5] Strain 1BD, in broth.

[6] M. 3, in broth.

[7] V. 1, in broth.

[8] Strain Horn, in broth.

[9] The substance is soluble in oil and in alcohol-water. It has been dissolved in alcohol and the alcoholic solution (0.5%) added to the culture medium. We have proved that in this amount the added alcohol had no action on bacterial growth. *In vivo,* we used a 2% oil solution. The bis(2-methylaminophenyl)disulfide is less toxic (10 mg is well tolerated) than bis(2-aminophenyl)disulfide (lethal dose is 10 mg/20-g mouse).

there are substances possessing chemotherapeutic activity sufficient to justify a thoroughly systematic study. The activity against *Leptospira* is particularly interesting. It seems that the *N*-alkyl derivatives of the *o*-amino series, which are unable to form ring compounds, such as inactive benzothiazoles, may be more active and have less toxicity than the corresponding nonalkylated derivatives. This follows from the behavior of bis(2-methylaminophenyl) disulfide. The activity of these compounds is connected with their oxidizing action. The bis(2-acylaminophenyl) disulfides, owing to their capacity for irreversible reduction (following cyclization to benzothiazoles) may in some cases constitute useful agents for the study of certain enzymatic systems.

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New Method for Blood Pressure Recording

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During the last decade there has been an ever increasing interest in electrical transmission methods for accurate measurement and recording of intra-arterial pressure. A number of methods have been described, e.g., photoelectric (*9*) and piezoelectric (*5, 6*) manometers, Clark reluctance manometer (Clark Pressure Capsule) (*7*), capacitance manometers (*1, 10*), and inductive (*8*) and resistance (*2, 4*) transmission methods.

In our investigations of the physiological effects on animals of high explosive blasts, we originally used a resistance-wire instrument (strain-gage) for the continuous recording of arterial blood pressure during the detonation (*3*). Since this method has several disadvantages, however, we have now developed an instrument in which the pressure variations are transmitted by a mechano-electronic transducer. So far as we know, this principle has not been used before in blood pressure recorders.

The instrument consists of a small pressure chamber with a thin silver membrane. The membrane acts directly upon the movable anode of the transducer tube (RCA 5734). The chamber and the short steel cannula which is inserted in the blood vessel are filled with a solution of 1% heparin in physiologic NaCl.

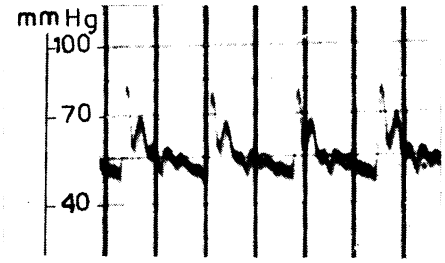

FIG. 1. A recording of the pressure in the carotid artery of a rabbit. The heavy vertical lines represent intervals of 1/10 sec. The curve is underdamped.

The transducer is connected to a pentode as a variable grid leak. The recording instrument (e.g., an oscillograph) is bridged in the anode circuit of the pentode. By using this way of coupling it is possible to make full use of the excellent frequency properties of the transducer. Another advantage is that the transducer has no other electric tension than the filament voltage and the grid voltage, which amounts to only a few volts.

The construction of the instrument is very simple and sturdy. Owing to the small dimensions of the transducer (length 20 mm, diam 8 mm) the whole instrument can be made very small and convenient. It requires only a slight amplification with one amplifier valve, or if the recording device is sensitive enough the amplification is not necessary at all. The instrument has a great range of frequencies and the phase displacement of the recordings is insignificant (Fig. 1). Owing to its low input impedance, the apparatus is quite insensitive to outside disturbances even if long feed lines must be used (as, for instance, in blasting experiments). The sensitivity of the instrument makes it possible to record fluctuations of pressure down to 1–2 mm of water. Supplied with a suitable membrane the apparatus can, therefore, be used even for recording venous pressure.

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