noted separately; the sum can be computed directly on the calculating machine.

The sum is in this case 20.9212 cm^2, and after division by the square of the magnification factor, one obtains the figure 3.2667 cm^2. This has to be divided by $2t(n_2-n_1) = 2 \times 5220 \times 66.33 = 692\,485$ sec. Consequently the diffusion coefficient is $4.717 \cdot 10^{-6}$ cm^2/sec. After recalculation from the temperature during the experiment, 21.1°, to 25.0°, and to infinite dilution as described by Gosting and Morris [12], one obtains $D_m = 5.254 \cdot 10^{-6}$ cm^2/sec. This figure differs by less than 0.1 per cent from the value obtained in the same experiment by the height-area method, and by 0.45 per cent from the value given by Gosting and Morris.

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On the Presence of a Tuberculostatic Factor in Organ Extracts from Cow *

Preliminary report

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The presence of a thermostabile substance in human urine, which dialyses through cellophane, and has a bacteriostatic and bactericidal effect toward tubercle bacilli has been reported earlier [1]. Furthermore, it could be shown that this substance was not identical with urea, hippuric acid, creatinine, urinary phenols, or salts. Experiments with urine from patients with tuberculosis of the kidney seemed to indicate that this type of urine had a weaker tuberculostatic effect than normal urine.

Because of this observation and because it had proved difficult to purify the tuberculostatic substance from urine, attempts were made to isolate a similar tuberculostatic substance from other sources. Bovine urine appeared to have an inhibitive effect toward tubercle bacteria of about the same order as human urine, and for this reason extracts from several organs from the cow were tested.

It could be shown that water extracts of spleen, lung, liver, kidney and muscle did have a tuberculostatic effect. The active substance from these extracts is similar to the urinary factor in that it is thermostabile and is adsorbed on activated charcoal. Furthermore, it can also be eluted from the charcoal with acetic acid.

* After the completion of this work I became aware a note by R. J. Dubos on the occurence of a tuberculostatic agent in animal tissues [*Am. Rev. Tuberc.* **63** (1951) 119]. The active factor had been extracted by acid alcohol from several organs of cattle, rabbits and guinea pigs.

A similar substance even seems to be present in bovine serum, and it can be separated from serum protein by dialysis.

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A Disregarded Complication in the Synthesis of β-Ketoesters by the Base Catalysed Acidolysis of Diethyl Acylmalonates

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The author has recently described a method for preparing β-ketoesters using a base catalysed acidolysis of diethyl acylmalonates according to the following equation [1]:

$$RCOCH(CO_2C_2H_5)_2 + RCO_2H \rightarrow RCOCH_2\,CO_2C_2H_5 + RCO_2C_2H_5 + CO_2$$

This reaction was found to give mixtures of β-ketoesters if the acyl group of the acid was not the same as that of the acylmalonate used [2].

This fact and some other observations have called my attention to the possibility that the β-ketoesters obtained are not quite pure even if both the acyl groups are the same. The main impurities resulting from the acyl group mobility [2] would be the corresponding β-diketone and diethyl malonate. The reaction leading to these products probably is:

$$RCOCH(CO_2C_2H_5)_2 + RCOCH_2CO_2C_2H_5 \rightarrow (RCO)_2CHCO_2C_2H_5 + CH_2(CO_2C_2H_5)_2$$
$$(RCO)_2CHCO_2C_2H_5 + RCO_2H \rightarrow RCOCH_2COR + RCO_2C_2H_5 + CO_2$$

In my first preparation of ethyl propionylacetate the possibility of an impurity in the form of diethyl malonate was fully realised and a test according to Breslow, Baumgarten, and Hauser [3] was made for this compound. This test was negative. These authors stated, that the complete solubility of the product in 5 % sodium hydroxide will show the absence of any appreciable quantities of diethyl malonate. However, such a statement is false as diethyl malonate itself is readily soluble in 5 % sodium hydroxide. Later experiments have shown, indeed, that the product was contaminated by appreciable quantities of diethyl malonate and dipropionylmethane.

It can be demonstrated that the ethyl diacylacetate is a probable intermediate product since ethyl diacetoacetate can be isolated in appreciable quantities from the reaction of diethyl acylmalonate and ethyl acetoacetate.

The second step in the reaction, the acidolysis of the ethyl diacylacetate with an organic acid is a well-known reaction for the preparation of β-diketones [4-6].

In order to see the extent to which these side reactions have proceeded in the preparations of the β-ketoesters reported in the previous publication, the products have been tested for the diketone and the diethyl malonate.

The synthesis of ethyl propionylacetate was repeated on a 2 mole scale. When the product was carefully fractionated through a very efficient Pyrex Widmer column, a forerun of 47 g b.p. 40—68°/7.5 mm was obtained. This fraction was precipitated with a solution of copper acetate, yielding the copper derivative of dipropionylmethane m.p. 212° corresponding to 31 g of dipropionylmethane b. p. 59°/9 mm, or a 12 % yield based on the diethyl propionylmalonate.

The forefraction was followed by a fraction boiling at 68—69°/7.5 mm and a fraction at 72—73°/7.5 mm together with a very small middle fraction b.p. 69—72°/7.5 mm. A determination of the refractive indices of the two main fractions showed that the low boiling product had a somewhat higher