

great service in connexion with drawings of skulls, as it is often desirable to show on profile drawings the depth and the shape of the orbit, palate, etc. The attachment for this work consists of a U-shaped metal pointer with parallel prongs, *G*, that is capable of horizontal and vertical adjustment and which is carried on an arm that is fixed in a runner at the side of the frame supporting the specimen; this runner is also capable of vertical adjustment. The width between the prongs is such that it is rather larger than half the maximum width of an average skull and the length of each of the prongs is precisely equal, so that the pointed tips are exactly in the same line of sight when viewed by the telescope. It follows that if the posterior prong is placed within a cavity and thereby hidden from view, then the anterior will indicate the actual depth of the cavity and by follow-

ing the movements of the anterior pointer with the telescope an outline of the cavity may be drawn.

It will be appreciated that all subsequent measurements can be made on the drawings instead of on the object. This is of great convenience, as it enables drawings to be submitted for examination rather than the actual object; in addition, any number of precisely similar drawings may be made.

Although the machine was primarily designed for drawing skulls, it can be used to advantage in making a series of true to size drawings of any object in any plane where accuracy of detail combined with speed are a consideration.

The illustrations show the complete apparatus with a skull mounted in position for drawing, and also two drawings—one of a skull (Fig. 2) and the other of a palate (Fig. 3)—obtained with the instrument.

## Recent Work on Vitamin D.

### II.

#### CHEMISTRY.

VARIOUS colour tests have been proposed for the detection of vitamin D, but none of those yet described is specific. Thus W. A. Sexton (*Biochem. J.*, vol. 22, p. 1133; 1928) has investigated the reaction obtained by heating a source of vitamin D with aniline hydrochloride in excess of aniline, when a red colour is produced. It was found that in addition to cod-liver oil and irradiated ergosterol, a similar colour was given also by unsaturated ketones, such as cholestenone, oxycholestenone, oxycholesterylene and carvone: with saturated ketones, such as cholestane-4-one or camphor, only slight darkening of the mixture was observed. The results suggest, however, that the vitamin, or an accompanying irradiation product of ergosterol, is ketonic in character. The phosphomolybdotungstic acid test is even less characteristic; though cod-liver oil gives a positive reaction, irradiated ergosterol is negative. Ergosterol itself, however, shows certain colour reactions which not only are useful for detecting its presence in mixtures, but also throw light upon its chemical structure as well as that of some other sterols.

O. Rosenheim (*ibid.*, vol. 23, p. 47; 1929) has found that when it is warmed with chloral hydrate or its chloroformic solution treated with a concentrated aqueous solution of trichloroacetic acid, an immediate red colour is produced which soon changes to a deep blue (passing through a green in the case of chloral hydrate). So little as 0.005 mgm. can be detected. The red colour is also given by cholesterylene,  $\psi$ -cholestene, allocholesterol, and allostosterol, and is therefore dependent upon the presence of the  $\Delta^{4,2}$  (or  $\Delta^{1,13}$ ) ethenoid linkage: it is probable that a coloured carbonium salt is formed. The colour is discharged by water or alcohol. The final blue stage observed in the case of ergosterol is presumably due to the presence of the third double bond. Oxycholesterol gives a gentian blue colour with the reagents, which, however, shows only the absorption band of oxycholesterol and not those of the ergosterol blue.

I. M. Heilbron and F. S. Spring (*ibid.*, vol. 24, p. 133; 1930), in further studies of the structure of sterols, have investigated the reaction with bromine: ergosterol and certain hydrogenated derivatives of this substance which do not give the red colour with trichloroacetic acid, when dissolved in glacial acetic acid show a green colour on addition of a solution of bromine in chloroform. It appears that this reaction depends upon the  $\Delta^{10,19}$  (or  $\Delta^{10,11}$ ) ethenoid linkage, which is inert to hydrogenation: all cholesterol derivatives, for example, are easily hydro-

genated completely; they fail to give the bromine test. Heilbron and Spring also direct attention to the fact that only those sterol compounds show selective absorption which contain the  $\Delta^{4,13}$  (or  $\Delta^{4,2}$ ) ethenoid linkage as one of two which are present in the molecule.

Since the discovery that ergosterol shows the highest antirachitic potency of any substance on irradiation with ultra-violet rays, search has been made for other substances which could be activated to a similar degree, but without success. Thus O. Rosenheim and T. A. Webster (*Biochem. J.*, vol. 22, p. 762; 1928) found that naturally occurring saturated sterols and artificially reduced sterols, cholesterol and sitosterol with one double bond, stigmasterol and cholesterylene with two were not activated on irradiation. Again, other unsaturated compounds containing three or more double bonds such as squalene, certain terpenes, sphingosine, and phrenosine were unable to replace ergosterol as precursors of vitamin D. Isomers of ergosterol, fungisterol from ergot and zymosterol from yeast, iso- and neo-ergosterol were equally impotent. The work on zymosterol was carried out by E. M. Hume, H. H. Smith, and I. Smedley-Maclean (*ibid.*, vol. 22, p. 980; 1928) and was complicated by the fact that the sterol still contained, after purification, as much as 5 per cent ergosterol, the impurity being detected by spectroscopic examination, since neither zymosterol nor fungisterol possesses any selective ultra-violet absorption. However, the biological test on rats showed that daily administration of  $\frac{20}{100000}$  mgm. irradiated ergosterol prevented rickets almost entirely, whilst the same slight activity was shown by doses of  $\frac{100}{100000}$  mgm. irradiated ergosterol and  $\frac{1}{50000}$  mgm. irradiated zymosterol: hence the effect produced by the latter can be ascribed to its ergosterol content.

In a further communication, Rosenheim and Webster (*ibid.*, vol. 22, p. 1426; 1928) give details of their examination of irradiated fungisterol for antirachitic potency as well as results obtained with two other sterols isolated from ergot. The fungisterol and one of the others contained small amounts of ergosterol as shown by spectroscopic examination and their biological activity after irradiation was low and certainly due to the presence of irradiated ergosterol. The third sterol showed no absorption bands, gave a negative colour test for ergosterol and was completely inactive after irradiation. All the evidence so far obtained, therefore, suggests that ergosterol is the only naturally occurring precursor of vitamin D.

In following the course of the reaction ergosterol  $\rightarrow$  vitamin D, it has been found that other products be-

sides the vitamin are formed: in fact, under conditions of irradiation such as are frequently employed, the latter forms only a small proportion of these products. Observation of the changes in the absorption bands of ergosterol forms a convenient method of following the course of the reaction, but potency tests are necessary before any particular band can be assigned to vitamin D, more especially since the changes vary to some extent according to the conditions of irradiation. By following these changes, Heilbron and his co-workers were first led to suspect that 'pure cholesterol' contained a contaminating provitamin, and finally to suggest that vitamin D was characterised by a band at 2470 Å.; the ergosterol bands are at 2935 Å., 2815 Å., and 2700 Å. Irradiation was carried out in alcoholic solution for 60 min. (I. M. Heilbron, E. D. Kamm, and R. A. Morton, *Biochem. J.*, vol. 21, pp. 78 and 1279; 1927; *J. Chem. Soc.*, p. 2000; 1927).

T. A. Webster and R. B. Bourdillon (*Biochem. Jour.*, vol. 22, p. 1223; 1928) found that irradiation with rays of wave-length longer than 2700 Å., by the use of a filter of alcoholic cobalt chloride which cuts off the shorter rays, did not alter the equilibrium reached and that exposure of the solution of ergosterol during irradiation to temperatures ranging from  $-18^{\circ}$  to  $77.8^{\circ}$  did not alter the potency of the final product, although at temperatures of  $-183^{\circ}$  and  $-195^{\circ}$  the preparations were markedly less active: these results suggest that the temperature coefficients of the changes causing production and destruction of the vitamin are similar and small and that both reactions are directly photochemical in nature. A highly active preparation was obtained by a short irradiation followed by removal of the bulk of the unchanged ergosterol with digitonin, evaporation to dryness and extraction in ether to remove traces of digitonin and ergosterol digtonide. The product was a glassy solid, melting at about  $30^{\circ}$  and much more soluble than the original ergosterol in organic solvents. When the absorption spectra of the irradiation products were studied, it was found that the first change was a marked increase in the absorption below 3000 Å., especially in the regions 3200-2900 Å., 2650-2500 Å., and 2900-2700 Å. More prolonged irradiation decreased the absorption and, at the same time, the activity. The authors conclude that vitamin D probably has an absorption maximum at 2800 or 2900 Å. and from it is formed a secondary product with an absorption maximum at about 2300 Å. Still longer irradiation of the products first obtained leads to complete disappearance of both absorption and activity. They point out that the presence of a band together with some activity in a preparation does not indicate that the band is to be attributed to the vitamin, unless a quantitative relation between such absorption and activity can be demonstrated.

Bourdillon and Webster and their co-workers consider the absorption spectrum of vitamin D also in a later paper (*Proc. Roy. Soc.*, B, vol. 104, p. 561; 1929). Their evidence suggests that three substances are formed in succession by the irradiation of ergosterol: the first (which is vitamin D) shows increased absorption as compared with ergosterol in the region 2500-3100 Å. with maxima at about 2800 Å. and 2700 Å. More prolonged irradiation results in decrease in the absorption in this region with a concomitant increase in that between 2300 and 2500 Å. and finally in almost complete disappearance of any absorption in the ultra-violet region. Quantitative comparisons of absorption spectra and antirachitic activity showed a roughly linear relation between intensity of absorption at 2700-3100 Å. and potency, so that presumably the substance first formed is the vitamin. The other two substances are inactive. When a filter cutting off all radiation below 2650 Å. was used, there was a marked decrease

in the formation of vitamin D, suggesting that shorter wave-lengths favour its production rather than its destruction.

The irradiation was carried out usually in alcoholic solution in the absence of oxygen: vitamin D was not easily oxidised in alcoholic solution, but very readily when exposed dry to oxygen at  $100^{\circ}$ . By calculations depending on the rate of destruction of ergosterol (determined gravimetrically) and the rate of production of absorption due to vitamin D, it was found that the purest preparations obtained probably contained about 55 per cent of the vitamin. The minimum dose detectable biologically was about  $2.5 \times 10^{-9}$  gm. or, in 14 days,  $3.5 \times 10^{-8}$  gm. If only half this was vitamin D, the smallest detectable dose of the pure vitamin would be  $1.9 \times 10^{-8}$  gm., a figure which agrees closely with Steenbock's and Coward's estimates.

E. H. Reerink and A. van Wijk (*Biochem. J.*, vol. 23, p. 1294; 1929) irradiated ergosterol in solution in hexane, exposing it to wave-lengths longer than 2750 Å. and to the wave-length 2540 Å. by interposing between the mercury arc and the solution filters of benzene solution or chlorine gas and potassium nitrate solution, respectively. Oxygen was rigidly excluded. With the long wave irradiation it was found that the absorption increased at first over the whole range, diminishing as irradiation proceeded in the long wave range. At 2820 Å., absorption decreased from the beginning, but at 2715 Å. it at first increased. It was possible to calculate that for the first 15 min. the conversion of ergosterol into vitamin D was the only reaction taking place and that in this time about half the ergosterol was converted. More prolonged irradiation resulted in slow destruction of the vitamin and disappearance of the above bands with the development of an ill-defined band at 2400-2500 Å. The product of a short irradiation was highly active and was obtained in the crystalline state, free from ergosterol, provided that oxygen was rigidly excluded during all manipulations: the crystals had a melting point below  $0^{\circ}$ .

Irradiation at 2540 Å. resulted in an increase in absorption over the whole range, including 2800 Å., the increase being much more marked than with the long wave irradiation: the absorption at 2930 Å. and 2820 Å. increased much more than that at 2715 Å.: further irradiation resulted in almost complete disappearance of the absorption. The vitamin was formed only during the first few minutes and was then rapidly destroyed: the most potent preparation had an activity of only about a tenth of that of the material obtained by long wave irradiation. In certain respects these results do not agree with those obtained by Bourdillon and Webster *et al.*, and it is obvious that further work is required before agreement is reached on the absorption spectrum of vitamin D, but the preparation of pure specimens appears now to be assured.\*

It may be pointed out that the stability of the vitamin may be influenced by the presence of accompanying compounds in a similar manner to the parent substance ergosterol. H. King, O. Rosenbeim, and T. A. Webster (*Biochem. Jour.*, vol. 23, p. 166; 1929) point out that although ergosterol itself is labile, as a contaminant of cholesterol it appears stable: they have, in fact, found it present in the cholesterol esters isolated from the brain of a mummy 1400 years old, both by colour test and also by obtaining an antirachitic product of the same order of potency as irradiated 'cholesterol', by its irradiation.

\* In a recent note in our columns (*NATURE*, vol. 125, p. 635; 1930), Bourdillon, Jenkins, and Webster state that they have now come to the conclusion that the absorption band at 2800 Å. is not that of vitamin D, but of a decomposition product: the vitamin shows low absorption at this wave-length.