

suitably planned if the results should be of any value.

Enough has been said to show that India should evolve her own methods both in the matter of planning, and in the conduct of

agricultural field experiments. A co-ordinating agency is of course necessary, and there is ample scope for mutual fellowship between the statistician and the agricultural experimenter.

### The Antianæmic Principle of Liver.

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THE use of liver in the treatment of pernicious anæmia constitutes a striking therapeutic advance of great importance. The idea must have first originated from Whipple and Robschiet Robbins<sup>1</sup> who in their search for blood regenerative foodstuffs found that of all the substances they investigated, liver was most potent as a hæmopoietic material. This discovery led Minot and Murphy<sup>2</sup> in 1926 to make a clinical trial on pernicious anæmia patients and as a result of their classical researches they obtained a remarkable improvement in the blood picture of the treated patients. Since then, there have been a number of investigations supporting their regimen and now we can completely restore the anæmic patients to normal health by administration of liver.

#### ETIOLOGY OF PERNICIOUS ANÆMIA.

Although the results of Minot and Murphy made it a logical conclusion that pernicious anæmia is a disease due to a dietary deficiency, there were also other theories prevalent to explain its cause. The accumulation of toxins in the body, the infectious disorders in the intestinal flora and the absence of the anti-hæmolytic substance were individually suggested as the causative factors. The exact etiological significance of the defective gastric secretion was first suggested by Fenwick<sup>3</sup> in 1880 and has, since then, been supported by other investigators. Goldhammer<sup>4</sup> has shown that gastric secretion is proportional to the red blood cells and in pernicious anæmia there is a sub-normal amount of gastric secretion also characterised by com-

plete anacidity. Castle and his coworkers<sup>5,6</sup> have shown conclusively that the stomach of a normal human being secretes some enzymic principle which, when allowed to react *in vitro* or *in vitro* with some substance present in the animal proteins of the food, produces the necessary antianæmic factor. The non-occurrence of this reaction in the body is believed to be a defect in the gastric digestion leading to pernicious anæmia. The secretory product is called the intrinsic factor and the substance derived from the food the extrinsic factor. The specific antianæmic principle thus produced is stored in liver from which it is elaborated as required by the bone marrow to produce the normal quota of erythrocytes.

The site and the mode of interaction of these two factors are not known. Their chemical nature is also obscure. The intrinsic factor is believed to be unrelated to either hydrochloric acid, pepsin, rennin or lipase. Klein and Wilkinson<sup>7</sup> have studied this intrinsic factor in considerable detail and have named it enzyme "hæmopoietin". Like most of the enzymes it is destroyed by heat. Griffith<sup>8</sup> has observed that its action is confined to  $P_H$  3.5-5.5. The food factor is, on the other hand, thermostable and is found to be present in beef-muscle, autolysed yeast, rice polishings, eggs and liver. It is not identifiable with any portion of vitamin B complex.<sup>9,10</sup>

Following the earlier papers of Castle and his coworkers, Sergius and Isaac,<sup>11</sup> and Wil-

<sup>1</sup> Whipple, G. H., and Robschiet Robbins, F. S., *Amer. J. Physiol.*, 1925, **72**, 395.

<sup>2</sup> Minot, G. R., and Murphy, W. P., *J. Amer. Med. Assoc.*, 1926, **87**, 470; 1927, **89**, 759.

<sup>3</sup> Fenwick, S., "On Atrophy of the stomach and on the nervous affections of the digestive organs." J. & A. Churchill, London, 1880.

<sup>4</sup> Goldhammer, S. M., *Proc. Soc. Expt. Biol. Med.*, 1926, **476**.

<sup>5</sup> Castle, W. B., and his coworkers, *Am. J. Med. Sci.*, 1920, **178**, 748-764.

<sup>6</sup> Castle, W. B., and his coworkers, *Am. J. Med. Sci.*, 1930, **180**, 305; 1931, **182**, 741.

<sup>7</sup> Klein, L., and Wilkinson, J. F., *Biochem. J.*, 1934, **29**, 168+.

<sup>8</sup> Griffith, W. J., *Biochem. J.*, 1934, **28**, 671.

<sup>9</sup> Diehl, F., and Kuhnau, J., *Deutsch. Arch. f. Klin. Med.*, 1933, **176**, 149.

<sup>10</sup> Lassen, H. C. A., and Lassen, H. K., *Am. J. Med. Sci.*, 1934, **188**, 461.

<sup>11</sup> Sturgis, C. C., and Isaac, R., *J. Am. Med. Assoc.*, 1929, **93**, 747.

kinson<sup>12</sup> showed that in some cases stomach preparations were as effective in bringing about the remission of the disease as liver itself. Snapper and Preez<sup>13</sup> have recorded instances where patients refractory to liver treatment responded very well to stomach preparations. This should not be taken to mean that stomach preparations will generally replace the liver therapy. In the instances cited it is likely that the gastric secretion has reacted on the proteins of the gastric tissues themselves to produce the required anti-anæmic principle.

Another collateral evidence for the inter-relationship of stomach and liver in the etiology of pernicious anæmia is furnished by the work of Goodman<sup>14</sup> and others who have shown that after gastrectomy in the pig, the antianæmic potency of the liver becomes progressively depleted and the animal becomes anæmic. But the total extirpation of stomach in man may not necessarily be followed by the development of pernicious anæmia even after some years. Evidence has recently been adduced by Meulengracht<sup>15</sup> that the active principle of the hog's stomach is present largely in the pyloric region where glands of a type closely resembling those of duodenum of both man and the hog are found. Further, the kidney also is known to contain some of the antianæmic principle and this circumstance might delay the onset of anæmia. This latter fact also renders difficult the postulation that disease of the liver may specifically produce an interruption in the metabolism which leads to the production of the active principle. Further work is needed to elucidate these interesting points.

#### TREATMENT.

For the oral administration, the patients require about half a pound of whole liver or its extract per day and the patient often takes an aversion to ingest such large quantities, particularly when the malady is complicated by nausea, vomiting, sepsis, and such other complications. Moreover, oral administration of the liver preparations to patients suffering from a severe type of pernicious

anæmia may not prove sufficiently rapid and the presence of strong auto-agglutinants in the patient's blood may render blood transfusion unsatisfactory. More recently, therefore, the extract has been purified free from proteins and such other impurities and rendered suitable for parenteral injections.<sup>16,17</sup> The effectiveness of the parenterally administered material is about 30-40 times as great as when given by oral route. The maximum increase in reticulocyte is also reached sooner than in the case of oral administration.

#### PREPARATION.

The full liver action of any preparation of liver extract can be secured only by the employment of unexceptional raw material, careful and skilled treatment of this material and a high degree of concentration of the active substances. Three methods are generally in vogue for the preparation of the active liver extract. The first is that employed by Cohn and his coworkers.<sup>18</sup> The raw minced liver is adjusted to a  $P_H$  5.2, extracted with water and the heat coagulable proteins separated by heating the extract to 70°C. It is then concentrated under vacuum, extracted with ether and finally precipitated by alcohol. The other method, described by Castle and Bowrie,<sup>19</sup> is to extract the well-minced liver with ice-cold water for 12-18 hours, remove the heat coagulable proteins and then concentrate the extract. The third method is described in *British Pharmacopœia* and consists in extracting the liver straightaway with 80% alcohol and concentrating the alcoholic extract. All these preparations are suitable for oral administration and for purposes of injection they are further purified by suitable methods.

The fluctuations in the clinical reports of the several preparations thus obtained emphasise the need for a complete study of the conditions for obtaining a highly potent extract.

#### NATURE OF THE ACTIVE PRINCIPLE.

Although much has been already learned about the therapeutic constituent of liver, its precise nature is still obscure. In the beginning, since iron and copper were known to accumulate in liver, the therapeutic value was

<sup>12</sup> Wilkinson, J. F., *Proc. Roy. Soc. Med.*, 1933, **26**, 1341.

<sup>13</sup> Snapper, I., and DuPreez J. D. J., *Nederland Tijdschr. Geneeskunde*, 1931, **75**, 29.

<sup>14</sup> Louis Goodman and others, *Proc. Soc. Expt. Biol. Med.*, 1935, **32**, 810.

<sup>15</sup> Meulengracht, E., *Proc. Roy. Soc. Med.*, 1935, **28**, 841.

<sup>16</sup> Wilkinson, J. F., *Lancet*, 1931, **221**, 791.

<sup>17</sup> Gansslen M., *Klin. Wochschr.*, 1930, **7**, 2099.

<sup>18</sup> Cohn Minot, Alles and Salter, *J. Biol. Chem.*, 1928, **77**, 325.

<sup>19</sup> Castle, and Bowrie, *J. Am. Med. Assn.*, 1929, **92**, 1830.

ascribed to these inorganic constituents. But very recently it has been definitely shown<sup>20,21</sup> that neither liver ash nor extraneous Iron or Copper produces in pernicious anæmia cases the well-known beneficial effect obtained with liver.

During their fractionation studies, Cohn and his coworkers<sup>18</sup> located the active principle in the filtrate from basic lead acetate. It could be precipitated by phosphotungstic acid giving a fraction containing 19% nitrogen. They carried out a number of qualitative tests and came to the conclusion that the active principle was not a carbohydrate, protein or lipid, a result which was also supported by Whipple and Robschiet Robbins. In a later paper, Cohn<sup>22</sup> and his coworkers concluded that the active principle is a nitrogenous base, the nitrogen in which exists, as in a secondary or tertiary amine, probably of the pyrrol or pyridine group.

West and Nicholas<sup>23</sup> showed that the best fractions prepared contained 12–14% nitrogen and amino nitrogen 20% of the total nitrogen increasing up to 40% after acid hydrolysis. Iron and phosphorus were absent from their preparations while sulphur was found in traces. The fractions gave a positive biuret, diazo and naphthol test, a weak Hopkin's test and a slight levorotation.

Felix and Fruhwein<sup>24</sup> have precipitated the active principle by adding to the aqueous extract mercuric sulphate in sulphuric acid solution. The active preparations contained at least 7% nitrogen, the amino nitrogen of which did not increase after acid hydrolysis. Their technique, however, suffers from the defect that the use of heavy metals renders the preparation partially inactive.

The researches of West and Howe<sup>25</sup> point to the fact that the active principle is composed essentially of two amino acids, Oxypyrrolin and Oxylglutamic acid, though their possible mode of linkage is still obscure.

The identification of the active principle with  $\beta$ -Hydroxyglutamic acid isolated from

the liver was not supported by later work.<sup>26</sup> Similar negative results were obtained with glutathione found in liver by Robert Fleming.<sup>27</sup> Several amino acids were tested for their therapeutic value and although in one or two cases like arginine and sodium glutamate good results were obtained,<sup>28</sup> it was found in general that none of the usual essential amino acids<sup>29,30,31</sup> was individually responsible for the therapeutic action of liver.

Very recently, in May 1935, Dakin and West<sup>32</sup> have obtained a very active preparation by precipitating the commercial liver extract first by Reinecke salt and then by saturation with ammonium sulphate. 30 mg. of their product caused a perceptible reticulo-cyte response in pernicious anæmia patients. The clinical activity of the product was readily abolished by exposure to cold 0.5 N alkali, by boiling for one hour with 0.5 N sulphuric acid or by salts of heavy metals. On hydrolysis, the active material yielded an amino-hexose and the following amino acids:—lysine (4.6%), arginine (13.5%), glycine (4.6%), leucine (20%), hydroxyprolin (10%), aspartic acid (17% and over), and glutamic acid (1.3%). It was also found that on hydrolysis with pepsin, the amino nitrogen did not increase; but on hydrolysis with erepsin, there was an increase in amino nitrogen while the product suffered from a loss of clinical activity. This shows clearly that the substance in question was a polypeptide. Its rough molecular weight was found to be 475–511 and optical rotation,  $(L)_D^{20} = -90^\circ$ .

In view of this probable simple polypeptide nature of the active principle, it appears possible to effect a concentration and purification of the extract by a process of adsorption followed by elution. Methods of simple ultra or electro-ultra filtrations of the extract through suitable membranes should also prove most useful in the purification of the active principle.

<sup>20</sup> Rudolph West, and Howe, M., *J. Biol. Chem.*, 1931, **94**, 611.

<sup>27</sup> Robert Fleeming, *Biochem. J.*, 1932 **26**, 461.

<sup>28</sup> Drabkin, D. L., and Miller, H. K., *J. Biol. Chem.*, 1931, **90**, 531.

<sup>29</sup> Keil, H. L., and Nelson, V. E., *Proc. Iowa Acad. Sci.*, 1933, **40**, 103.

<sup>30</sup> Elvehjem and others, *J. Biol. Chem.*, 1931, **93**, 197.

<sup>31</sup> Giorgio Dominici, and Fansta Penati, *Minerva Med.*, 1931, **11**, 413.

<sup>32</sup> Dakin, H. D., and West, R., *J. Biol. Chem.*, 1935, **109**, 389.

<sup>20</sup> Elden and McCann, *Proc. Soc. Expt. Biol. Med.*, 1927-28, **25**, 746.

<sup>21</sup> Jackson, H., Klein, L., and Wilkinson, J. F., *Biochem. J.*, 1935, **29**, 330.

<sup>22</sup> Cohn, E. J., McMeekin, T. L., and Minot, G. R., *J. Biol. Chem.*, 1930, **87**, xlix.

<sup>23</sup> Rudolph West, and Nicholas, E. G., *J. Am. Med. Assoc.*, 1929, **91**, 867.

<sup>24</sup> Felix, K., and Fruhwein H., *Z. Physiol. Chem.*, 1933, **216**, 173.

<sup>25</sup> West, R., and Howe, M., *J. Biol. Chem.*, 1930, **88**, 427.

## ASSAY OF THE POTENCY.

In spite of the several attempts, there has been no simple and satisfactory method of assaying the potency of liver preparations in some measurable units. The development of a suitable method of biochemical assay would be of immense value not only for the progress of further investigations like the comparative study of the various livers for their antianæmic potency but also for the standardisation of dosages. The only reliable method that is now available is by an actual trial upon pernicious anæmia patients and noting the increase or otherwise of the erythrocytes and reticulocytes. But human cases not being generally available for experiment, progress in this direction is bound to be slow. Attempts at developing a simpler method by using animals have met with little success.

McGowan<sup>33</sup> has shown that pernicious anæmia in fowls accompanied by myelocytic proliferation of liver closely resembles the disease in human beings. He used 19 leghorn fowls, subjects of spontaneous attack of disease to determine the minimum dose of liver extract required to produce significant change in the blood picture. The extract was given orally as well as intraperitoneally. Though apparently good results were obtained, the method is only qualitative and needs to be confirmed.

The work of Vaughan *et al*<sup>34</sup> has shown that the administration of substances capable of alleviating pernicious anæmia in man produces a response in healthy pigeons similar to that occurring in clinical cases, thereby providing a biological test for the potency of these substances. Relatively pure liver preparations known to be effective in pernicious anæmia administered either by mouth or by intravenous injection gave consistent response by way of rapid increase in the circulatory reticulocytes and a pronounced gain in weight. Continuing the work of these authors, Edmunds *et al*<sup>35</sup> and Peabody and Neale<sup>36</sup> have outlined a method for testing the clinical value of liver preparations by observing their action on healthy pigeons. But when

Wills,<sup>37</sup> Heiman *et al*<sup>38</sup> and Gurd<sup>39</sup> tried this method, they found spontaneous fluctuations even in control animals thus giving inconclusive results.

Jacobson<sup>40</sup> has attempted to use guinea pigs for the assay of the antianæmic factor and has even defined the minimum quantity (0.6 gm./Kgm. body wt.) as one guinea pig (G.P) unit.

Deusberg and Koll<sup>41</sup> adopted an *in vitro* method for testing the liver extracts for their potency. The active substance when added to hæmolysed human blood, destroys the active hæmoglobin spectrum and methamoglobin takes its place. The interfering substances like iron, sulphæmoglobin can be checked by adding suitable reagents. Deutsch and Wilkinson<sup>42</sup> have recently shown, however, that this method cannot be relied upon since no correlation can be obtained between the clinical activity and methamoglobin production. If it is possible to develop this method, it would no doubt offer advantages over other clinical tests; it would give quick results even with small quantities of the substances.

The production of the actual pernicious anæmia in animals has not been achieved so far. Since there are two factors involved in the etiology of pernicious anæmia it would perhaps be possible to produce the disease by controlling one or the other of these two factors. The most logical way of achieving this, would probably be, by means of a suitable diet. McCarrison<sup>43</sup> has shown that different diets would produce different influences on the gastro-intestinal tract. It remains to be proved by future experiments whether a defective gastric secretion thus produced would not lead to the production of pernicious anæmia. Miller and Rhoads<sup>44</sup> have tried a certain diet on guinea pigs and have produced a disease corresponding

<sup>37</sup> Wills, *Brit. J. Exp. Path.*, 1932, **13**, 172.

<sup>38</sup> Heiman, Connery and Goldwater, *Am. J. Med. Sci.*, 1934, **188**, 343.

<sup>39</sup> Gurd, M. R., *Quart. J. Pharm. and Pharmco.*, 1935, **8**, 39.

<sup>40</sup> Jacobson, B. M., *Science*, 1934, **80**, 211; *J. Clin. Invest.*, 1934, **13**, 714.

<sup>41</sup> Deusberg, R., and Koll, W., *Arch. f. Exper. Path.*, 1931, **162**, 296.

<sup>42</sup> Deutsch, W., and Wilkinson, J. F., *Brit. J. Expt. Pathol.*, 1935, **16**, 33.

<sup>43</sup> McCarrison, Robert, "Studies in Deficiency Disease," Henry Frowde, Holder and Stoughton, London, 1921.

<sup>44</sup> Miller, D. K., and Rhoads, C. P., *J. Clin. Invest.*, 1935, **14**, 153.

<sup>33</sup> McGowan, J. P., *Arch. Intern. Med.*, 1932, **49**, 26.

<sup>34</sup> Vaughan, J. M., Muller, G. L., and Zetzel, *Lancet*, 1930, **218**, 1062.

<sup>35</sup> Edmunds, Bruckner, and Fritzell, *J. Am. Pharm. Assoc.*, 1933, **22**, 91.

<sup>36</sup> Peabody, W. A., and Neale, R. C., *J. Am. Pharm. Assoc.*, 1933, **22**, 231.

to canine Black Tongue. Specific improvement is noticed in such cases when liver extract is administered. It should, therefore, be a very fruitful line of investigation to try and produce pathological conditions in animals similar to pernicious anæmia by controlling the diet and then test the effect of liver preparations on them.

Another promising line of enquiry into the assay of the antianæmic materials would probably lie in estimating one or two of the component amino acids of the potent

polypeptide before and after its hydrolysis. Since the purest preparation of Dakin and West has been shown to contain arginine to the extent of 13.5% and aspartic acid, more than 17%, it should be possible to obtain an idea of the concentration of the active polypeptide by estimating one or both the above constituents. Work in this direction might yield results of great practical utility in the assay of the active principle.

### Economic Ornithology in India.

By Sálím Ali.

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A CHARGE that has been preferred against ornithologists in India, perhaps not altogether without reason, is that they have been, and are, far too busy "classification-mongering", *i.e.*, quibbling over morphology and taxonomy, to bother about the *living* bird. Upto a point it may be argued in their defence that before biological studies on any group of animals can be undertaken it is essential that the forms belonging to that group should first be properly classified and made cognisable. But while acknowledging the stirring work done in this direction by ornithologists—wholly European—during the last century and still being carried on by their torch-bearers to-day, there is no doubt that the various other aspects of Indian ornithology have suffered a corresponding neglect.

The Indian Empire encompassing as it does an infinite diversity of climates and physical features—ranging from the eternal snows of the Himalayan peaks to the torrid deserts of Rajputana and Sind—contains an avifauna that for richness and variety can scarcely be rivalled by areas of similar size elsewhere in the world. The total number of species and sub-species so far described is just over 2,350 (including about 350 winter visitors) and more are being added to the list as fresh material from insufficiently worked areas or groups becomes available. Notwithstanding this prodigality of material, our knowledge of the living bird in India is surprisingly meagre. Beyond the barest facts about the nests and eggs of most (but still not all) of them, we know practically nothing concerning their breeding biology. The study of migration—one of the most

engrossing of bird activities and one that has stirred Man's wonderment from the earliest times—is here still in its veriest infancy compared with the researches and the strides being made in Western countries. Bird ecology, despite the vast natural facilities, remains practically an untouched and virgin field, while Economic Ornithology—an aspect of bird study that should have been, if for purely materialistic reasons, one of the foremost to receive attention in an agricultural country like India, has not even been scratched on the surface.

Besides being a source of direct food supply to millions of human beings in this country, it is little realised that wild birds stand in a class by themselves—second only, if at all, to predaceous and parasitic insects—as destroyers of, and natural checks on, harmful insect pests and other vermin, and as agents in the cross-pollination of flowers and the dissemination of seed. Directly or indirectly they exert their influence in practically every branch of human industry.

Economic Ornithology is the science that concerns itself with striking a precise balance between the damage caused by birds to Agriculture, Horticulture, Forestry and other human interests as against the active benefits they confer in less obvious ways. An increasing amount of importance is being attached in recent years to this science in Europe and America with excellent and far-reaching results. In the United States there is a well-organised department carrying on continuous and intensive research work on the life-histories of birds with special reference to their food and feeding habits under the Bureau of Biological Survey, a subsidiary