pituitary adrenal mechanism is still a matter for speculation.

At this stage, however, it is sufficient to note that the eosinopenic response to A.C.T.H. and oral cortisone in normal subjects can be usefully compared, and it remains to be seen whether the application of a combined A.C.T.H.-cortisone response test to patients with rheumatic, endocrine, and other diseases will give information of practical value.

Summary

A convenient modification of Dunger's technique for estimating the number of circulating eosinophils has been described and shown to be accurate enough for experimental purposes. By the use of this technique the eosinopenic responses to single doses of oral cortisone and intramuscular A.C.T.H. have been studied in some detail in four normal subjects. It has been shown that the time relations of the responses to these two hormones are remarkably similar, but that there may be considerable individual variations in responsiveness to either cortisone or A.C.T.H., or both.

The eosinopenic response to various A.C.T.H. preparations and to stress have also been compared, and throughout these studies the differences of individual responsiveness of our subjects have been maintained. Three patients with panhypopituitarism showed an eosinopenic response to oral cortisone which was exaggerated and prolonged. In spite of the many factors which may influence eosinopenic responsiveness it is suggested that a study of the eosinopenic response to 50 mg. of oral cortisone, added to the already well-established A.C.T.H. response test, might provide a useful guide to therapy with these hormones in the individual concerned, and might possibly give some indication of the state of activity of the individual's own pituitary-adrenal mechanism.

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Speaking at a meeting of the Society of Public Analysts recently about the chemical quality of milk Dr. J. G. Davis said that it was frequently stated that the overall composition of milk in this country had deteriorated during the last few years. It was difficult, if not impossible, to prove this contention by adequate scientific evidence for the whole country, and the reasons for this were discussed. A review of existing information was given, and data received from a number of public analysts on the composition of milk as sold over the period 1900-51 were presented. Not all records showed a fall in composition over a long period, and yearly and regional variations may be of the same order as the fall which it was claimed has taken place.

EOSINOPHIL COUNTING: A MODIFICATION OF PILOT'S METHOD

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The use of A.C.T.H. and cortisone entails the quantitative determination of the circulating eosinophils at frequent intervals as one of the pathological tests employed as a potential measure of the efficacy of the drugs. The method most commonly in use is that originally described by Randolph (1944). The object of this paper is to call attention to the diluting fluid evolved by Pilot (1950) and to the modification of that fluid it has been necessary to make in this laboratory.

Pilot investigated the counting fluids that had already been described. From them he selected as the best those of Randolph (1944) and Rud (1947), and combined the better properties of each in his diluent. Randolph's fluid, a phloxine-methylene-blue stain in propylene glycol, has certain advantages. The glycol base renders the red blood cells relatively non-refractile, and acts as a vehicle for both acid and basic stains, while, being viscous, it does not evaporate quickly. There is no deterioration of the count obtained when the fluid and blood are left in the pipette overnight. All the leucocytes, however, are stained, and a high-power objective is necessary for differentiation of the eosinophils. The methylene blue and phloxine components of the stain must be kept separate until just before use, for after they have been mixed for four hours precipitation of the dyes occurs and with it a loss of differential staining detail. The Rud fluid, a magdala-red stain in acetone, likewise has an advantage. By the use of a small amount of sodium carbonate (0.1%) all leucocytes other than the base-resistant eosinophils are lysed. Its disadvantage is the use of acetone as the vehicle. As this substance is highly volatile, the chambers must be flooded at once on shaking and kept in a moist Petri dish for 15 minutes before making the count.

Pilot decided to use the concentration of base employed by Rud in the propylene glycol vehicle of Randolph. Since the leucocytes other than the eosinophils were lysed, only a single stain was necessary, and no differential count was involved. As stains he used phloxine (0.1%), eosin (0.05%), and magdala red (0.05%), all with equally good results. His diluting fluid he compared with those of Randolph (1944), Rud (1947), and Dunger (1910), employing double counts in a series of 1,000 observations on human blood, with results more nearly constant than those obtained with the other fluids.

The stain he suggested is obtained by mixing the following ingredients: 50 ml. propylene glycol, 40 ml. distilled water, 10 ml. 1% phloxine in water, and 1 ml. 10% sodium carbonate in water. It is necessary to wait for 15 minutes after collection of the count to allow for proper lysis and staining, these occurring in either pipette or chamber.

Investigation

We have investigated Pilot's fluid in this laboratory for a period of four months, and the results obtained by its use are comparable to those obtained by the use of Randolph's fluid. The fluid possesses all the qualities claimed by Pilot. The use of a low-power objective is adequate, for the eosinophils alone are stained and visible, and the counting is thereby both easy and rapid. Pilot claims that there is no perceptible drop in counts done within three hours of collection and that there is an average drop of 35% in the count when the pipette lies for 24 hours. It has been found here that the timing is much more critical than he suggests. The accompanying table shows the results of standing the pipettes for four hours, and a considerable variation in the

When the delay between collection of the blood and counting was less than one hour it was found that the eosinophils were spread homogeneously throughout the chambers, and the totals in each approximated closely. When, however, the delay exceeded one hour the scatter of cells was not even, and fallacies resulted. To investigate the error entailed by a delay in counting, a period of four hours was selected as being the maximum period likely to elapse between collection and counting in a laboratory where routine haematology was more imperative and yet where the eosinophil counts had to be collected four hours after the morning injection of either A.C.T.H. or cortisone. The error is shown in the table. Counts were done from duplicate samples of blood immediately after collection and after a delay of four hours.

Table Showing the Effects on Eosinophil Counts of Standing Four Hours, Using Pilot's Fluid, With and Without the Use of Heparin

Case No.	Procedure	3rd Drop from Pipette					7th Drop from Pipette					12th Drop from Pipette					Mean Chamber Count	Eosinophil Count per c.mm.
		Chamber Counted				Total	Chamber Counted				Total	Chamber Counted				Total		
		1	2	3	4		1	2	3	4		1	2	3	4			
1	Immediate counting ..	14	13	12	13	52	13	12	11	13	49	13	13	12	12	50	12.6	140
	Stood 4 hours unmodified ..	8	10	7	8	33	10	5	6	5	26	4	5	4	8	21	6.7	74
	Stood 4 hours heparinized ..	12	11	15	13	51	10	14	11	13	48	12	10	12	14	48	12.3	137
2	Immediate counting ..	32	34	31	32	129	31	30	30	32	123	30	29	32	30	121	31.1	345
	Stood 4 hours unmodified ..	27	18	37	30	112	19	27	28	19	93	28	25	48	18	119	27.0	300
	Stood 4 hours heparinized ..	33	34	28	31	126	32	38	35	29	134	32	29	31	29	121	31.8	353
3	Immediate counting ..	30	32	32	30	124	29	30	31	32	122	30	33	32	30	125	30.9	343
	Stood 4 hours unmodified ..	17	14	20	19	70	11	19	16	13	59	22	16	27	26	91	18.3	203
	Stood 4 hours heparinized ..	33	31	29	31	124	30	35	30	31	126	28	33	30	32	123	31.1	345
4	Immediate counting ..	28	31	29	30	118	28	29	31	33	121	28	28	26	31	113	29.5	327
	Stood 4 hours unmodified ..	35	33	50	29	147	23	14	53	25	115	19	24	41	21	105	30.6	340
	Stood 4 hours heparinized ..	32	28	29	34	123	29	30	33	28	120	30	32	27	29	118	30.3	336
5	Immediate counting ..	10	9	9	10	38	8	9	11	13	41	9	8	13	11	41	10.0	111
	Stood 4 hours unmodified ..	4	3	3	5	15	5	3	5	3	16	2	8	5	4	19	7.1	79
	Stood 4 hours heparinized ..	11	9	10	9	39	10	10	10	13	43	9	10	9	9	37	10.0	111
6	Immediate counting ..	48	53	47	50	198	52	48	51	51	202	47	49	56	57	209	50.8	564
	Stood 4 hours unmodified ..	41	28	31	34	134	36	33	56	39	164	31	54	29	41	155	37.8	420
	Stood 4 hours heparinized ..	51	49	55	56	211	50	54	47	53	204	51	55	53	46	205	51.7	574
7	Immediate counting ..	24	20	21	27	92	24	23	27	26	100	23	25	24	20	92	23.7	263
	Stood 4 hours unmodified ..	2	4	4	4	14	4	5	4	3	16	2	5	6	10	23	4.4	49
	Stood 4 hours heparinized ..	20	24	26	28	98	26	25	22	26	99	25	23	24	22	94	24.3	270
8	Immediate counting ..	2	2	3	3	10	2	4	3	2	11	3	2	2	4	11	2.6	29
	Stood 4 hours unmodified ..	0	0	2	2	4	4	6	0	2	12	3	0	0	1	4	1.7	19
	Stood 4 hours heparinized ..	2	3	3	3	11	3	3	4	4	14	3	2	3	2	10	2.9	32
9	Immediate counting ..	25	27	25	29	106	27	29	30	25	111	24	27	26	24	101	26.5	294
	Stood 4 hours unmodified ..	12	13	20	21	66	15	24	14	15	68	20	16	20	24	80	18.8	209
	Stood 4 hours heparinized ..	27	26	28	25	106	26	24	27	28	105	29	26	27	25	107	26.5	294
10	Immediate counting ..	12	14	14	13	53	16	12	14	14	56	14	12	16	15	57	13.8	154
	Stood 4 hours unmodified ..	6	8	10	7	31	7	12	6	8	31	9	8	8	10	35	8.0	89
	Stood 4 hours heparinized ..	13	14	12	13	52	13	17	12	12	54	12	14	13	12	51	13.1	145

The figures in black indicate that clumps of cells were visible in the counting chamber.

counts has been noticed after a stand of only one hour. The table shows the results obtained in 10 unselected cases taken from the total of 36 cases so investigated.

The routine procedure followed has been to collect blood from a fingerprick in the proportions indicated by Pilot (blood to the 1 mark in a white-cell diluting pipette, diluted to the 11 mark with fluid), the blood and diluting fluid being mixed thoroughly by rotation during and after collection. Before counting, the pipette has been shaken for two minutes in a mechanical shaker of the type described by Sanders *et al.* (1950). Four chambers of a Neubauer haemocytometer were flooded, and counts made of the eosinophils contained in all nine squares of each. The number of eosinophils contained in a cubic millimetre is calculated by multiplying the average number of cells per chamber by 11.1. It is clear from the table that appreciably more accurate results are not obtained by counting eight or more chambers rather than four.

The possible cause of this error was obvious microscopically. When the pipette had stood for four hours, or in the majority of cases for only one to two hours, small aggregations of cells were visible in almost every chamber examined. These clumps appeared to be composed of ghost leucocytes apparently bound together by a finely meshed stroma, and they contained varying numbers of eosinophils. The count obtained from such chambers depended upon whether or not a relatively large clump containing many eosinophils lay within the counting area of the chamber. If it did, the count was raised above that obtained from the immediately counted preparation: if it did not, the count might be greatly lowered.

That the deviation of the results was due to insufficient mixing of the blood and diluting fluid was eliminated by counting four chambers from the third drop from the shaken pipette, four from the seventh drop, and four from the twelfth drop, these being roughly the specimens

obtained from the bottom, middle, and top of the bulb. The table shows the scatter of cells to have been even throughout the pipette in every case, both in chambers flooded from pipettes immediately examined and in those which had been allowed to stand for four hours.

That these clumps of cells could not be broken up by prolonged shaking was proved in four cases by shaking a duplicate pipette for ten minutes, the microscopical appearances remaining unchanged. The homogeneity of the counts obtained from 12 chambers flooded within 30 minutes of collection and the absence of clumping in all counts thus examined gave sufficient proof that clumping was not due to insufficient mixing of blood and diluting fluid at collection. To check this, four duplicate pipettes were shaken for two minutes immediately after collection and again after standing for four hours. Clumps still appeared in the majority of chambers examined.

In an attempt to prevent this error, due possibly to the normal clotting of the blood within the pipette, heparin was added to the diluting fluid in a concentration of one unit per millilitre. The table shows that this addition completely eliminated the error, the counts so obtained on blood which had stood for four hours being closely comparable to those obtained on immediate counting. Nor were clumps visible microscopically in the preparations.

Such clumping does not follow the standing of pipettes when Randolph's fluid is used. Though the addition of heparin eliminates the error when using Pilot's fluid, it is unlikely, therefore, that a clotting mechanism alone is responsible. The answer possibly exists in the pH difference between the two stains. The pH of Randolph's fluid is 7.4, that of Pilot's fluid 10.55. The alkaline pH of the latter would allow of possible partial denaturing of the blood proteins, fibrin being the first partially to precipitate with pH variation. The use of buffer solutions other than sodium carbonate to produce a pH of 10.55 brought about similar clumping of the cells on standing.

Two further crude tests have been employed to establish that the stroma which binds the cells on standing is fibrin. First, streptokinase (Cathie, 1949) has been added to the diluting fluid in a concentration of approximately four units per millilitre. This experiment has been done in six duplicate tests. The counts obtained after a delay of four hours when using such a modified stain are, within experimental error, those obtained following the use of heparin and those resulting on immediate counting of unmodified fluid preparations. Secondly, 1 ml. of blood has been added to 9 ml. of diluting fluid and the mixture allowed to stand for four hours. Flooded over a white surface, the fluid shows macroscopic clots which, when gathered from several specimens and sectioned, appear to consist of a stroma of material staining as fibrin enmeshing eosinophils and other leucocytes. A more detailed investigation of this problem is proceeding.

Summary

Attention is drawn to Pilot's fluid for the enumeration of eosinophils. Counting is much easier and quicker than with Randolph's fluid, while the disadvantage of Rud's fluid is also overcome.

A source of error in the use of the fluid is the clumping of leucocytes on standing. This may be eliminated by the addition of heparin to the fluid in the concentration of one

unit per millilitre. There is some evidence that this clumping is due to the formation of fibrin within the pipette.

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ADDISON'S DISEASE WITH ASSOCIATED POLYARTHRITIS AN AETIOLOGICAL STUDY OF THE ARTHRITIS SYNDROME

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In view of the therapeutic response to cortisone (compound E) in Addison's disease and in rheumatic diseases some correlation between these two disorders might be expected. In the literature, however, the association of the two diseases is notably infrequent. Bauer *et al.* (1951) could find no record of active rheumatoid arthritis in association with untreated Addison's disease.

Addison's disease is undoubtedly an endocrine disorder, but the possibility that the rheumatic diseases are fundamentally due to an endocrine disorder has not been demonstrable by clinical, laboratory, or pathological investigation. Some authors (Dejean, 1947; de Gennes *et al.*, 1947; Laroche, 1947a, 1947b) have reported Addison's disease with rheumatic joint lesions in cases under treatment with deoxycortone acetate (D.C.A.).

Selye (1949) noted that rats treated with large doses of D.C.A. often developed joint lesions which had histological changes characteristic of rheumatic arthritis and periartthritis. These lesions occurred in only a percentage of the rats, although some rheumatic manifestations were present in all. In some rats in which the arthritis appeared within a few weeks it was noted that the arthritic lesions cleared in spite of continued administration of D.C.A. Rarely, the arthritic lesions progressed to lesions comparable to those seen in rheumatoid arthritis. Selye found that if rats were adrenalectomized before the administration of D.C.A. they were more susceptible to these toxic effects of D.C.A. on the joints. He observed that cortisone relieved the arthritis. He stated that the significant aetiological factor in the genesis of the rheumatic joint lesions is an imbalance between the mineralo-corticoids of the adrenal cortex and the gluco-corticoids.

The following case is presented on account of the current interest in the aetiology of arthritic lesions.