colostrum, and were then kept for 3 weeks on the standard diet. Of the six calves deprived of colostrum five died. All the other calves survived.

3. The survivors scoured severely, and gained little weight during the experimental period.

4. Heating to 63° for 30 min. did not impair the protective value of the non-fatty fraction of colostrum.

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The Nutritive Value of Colostrum for the Calf

3. Changes in the Serum Protein of the Newborn Calf following the Ingestion of Small Quantities of the Non-fatty Fraction

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The serum of the newborn calf contains little or no euglobulin and pseudoglobulin I (Howe, 1921*b*). A marked increase in these protein fractions results from the ingestion of colostrum. Similarly, more recent studies, particularly by E. L. Smith (for a summary see Smith, 1948), have demonstrated the absence from the serum of the newborn calf of electrophoretically slow-moving globulins and their appearance following the intake of colostrum. These globulins are identical with the 'immune lactoglobulins' present in colostrum. The properties of these fractions differ slightly but significantly from those of the γ- and T-components found in the serum of the adult bovine (Smith, 1948). With calves receiving large amounts of colostrum the appearance of the 'immune lactoglobulins' in the blood stream can be demonstrated with ease, but it was not known whether the small quantities of the non-fatty fraction of colostrum given in the main experiment of Aschaffenburg, Bartlett, Kon, Walker, Briggs, Cotchin & Lovell (1949) would cause detectable changes in the serum proteins of the calves.

METHODS

Sampling of blood

Blood samples were obtained before the first feed and on the 4th day of life from every calf used in the main experiment of Aschaffenburg *et al.* (1949), with the exception of a few animals at the beginning of the experiment. The choice of the 4th day for the second sampling was dictated by reasons unconnected with this study. The delay was of little importance, as the rapid transfer of colostral proteins into the blood stream was found to be followed by a period of days during which the protein composition of the serum remained almost constant.

Nitrogen partition in blood serum

Total nitrogen and euglobulin nitrogen were determined by Kjeldahl analyses. The euglobulin was precipitated from 2 ml. of serum by the addition of 20 ml. of saturated sodium chloride solution followed by saturation with solid sodium chloride at room temperature. This procedure was preferred to the tedious precipitation with sodium sulphate at 37°. Howe (1921 *a*) showed that the results obtained with these two methods of salting-out are almost identical.

Globulin-turbidity test for blood serum

Nitrogen determinations, even as outlined above, are time-consuming, and a simpler and quicker test for the presence of 'immune lactoglobulins' was developed.

General

Kunkel (1947) described a turbidimetric method for the detection and estimation of pathological increases in the γ-globulin level of human serum, such as occur in certain diseases of the liver. The method consists in the measurement of the degree of turbidity developed when highly dilute metal-salt solutions, e.g. of copper sulphate or zinc sulphate, are allowed to react with serum. According to Kunkel, this reaction is specific for γ-globulin. As the 'immune lactoglobulins' are similar to, though not identical with, γ-globulin, the possible application of a test based on Kunkel's principle to calf serum was investigated. It was found that an approximately tenfold increase in the concentration of metal salt led to conditions under which no turbidity occurred with the serum of calves deprived of colostral proteins, whereas turbidities of an intensity reflecting the amount of proteins ingested developed after the calves had been given colostrum or its non-fatty fraction.

Method

Serum (0·1 ml.) is washed out from a blood pipette into 1 ml. of neutral distilled water (*a*) contained in a test-tube. Five ml. of a solution of 250 mg. of $ZnSO_4.7H_2O$/l. (*b*) are run in, and the contents of the tube are well mixed. The tube is left for 1 hr. (*c*), and the degree of turbidity is then determined in a photoelectric absorptiometer (*d*) or other suitable instrument.

Notes. (*a*) It is unnecessary to buffer the system if care is taken that the distilled water used in the test and in making up the zinc-sulphate solution is neutral. If necessary, the pH may be adjusted by the addition of dilute acid or alkali to the water.

(*b*) Solutions of zinc sulphate rather than of copper sulphate were chosen, as they remain clear and stable for longer periods.

(*c*) The turbidities develop rapidly at first, and then at a decreasing rate. The reaction is not quite complete after 1 hr., but this period was arbitrarily chosen as convenient and satisfactory for comparative purposes.

(*d*) The turbidities were determined in the 2 cm. cell of a Miller photoelectric absorptiometer (Morris, 1944), using the Ilford red filter no. 608. A reagent blank (no serum) was used to set the instrument to full-scale deflexion, and the turbidity readings were recorded as log-scale divisions, one division being equal to an extinction of 0·002.

RESULTS

General

The results of the nitrogen determinations and of the turbidity tests are given in Table 1 which also includes some data for calves that had received large amounts of colostrum by suckling their dams.

Total nitrogen

There was an increase in the total nitrogen of the serum of the four calves that had been given 400 ml. of the non-fatty fraction, whereas no consistent changes resulted from the other treatments.

Euglobulin nitrogen

According to Howe (1921*b*), the serum of the newborn calf does not contain euglobulin. Contrary to this statement, measurable amounts of euglobulin (mean: 33 mg./100 ml.) were, with the one exception of calf no. 18, found in every serum obtained from newborn calves. With the calves that received no colostrum, a reduction in serum euglobulin was observed on the 4th day when the calves were usually moribund. Where 80, 200, or 400 ml. of the non-fatty fraction were given, the changes in the euglobulin level were not consistent. Euglobulin nitrogen determinations were, therefore, not a valuable criterion in deciding whether or not a newborn calf had received small quantities of colostral proteins.

Globulin-turbidity test

The simple turbidity reaction proved more sensitive: a clear-cut positive result was obtained with the serums taken on the 4th day from all calves that had been given 200 or 400 ml. of the non-fatty fraction; 80 ml. was not sufficient to cause noticeable turbidity. Pl. 1 shows typical results for calves in one block given different quantities of the non-fatty fraction. The result for a calf that had suckled its dam is also shown. As the

Table 1. *Changes in the serum protein of newborn calves following the ingestion of small quantities of the non-fatty fraction of colostrum*

Quantity of non-fatty fraction given (ml.)	Calf no.	Total nitrogen (mg./100 ml.) Before feeding	4th day	Change	Euglobulin nitrogen (mg./100 ml.) Before feeding	4th day	Change	Globulin turbidity (log-scale divisions) Before feeding	4th day	Change	Remarks
0*	1	(1264)	(1040)	(−224)	—	(223)	—	—	(400)	—	Calf had evidently suckled (replaced)
	2	773	770	−3	41	22	−19	2	—	—	—
	3	664	802	+138	28	71	+43	3	218	+215	Only survivor in this group, likely to have suckled
	4	718	808	+90	33	18	−15	0	0	0	—
	5	739	722	−17	26	20	−6	0	0	0	—
	6	747	650	−97	51	21	−30	5	6	+1	Sampled on 2nd day; calf dying
80*	7	730	736	+6	72	84	+12	68	95	+27	Calf may have suckled
	8	653	683	+30	26	37	+11	0	7	+7	—
	9	761	725	−36	31	38	+7	0	10	+10	—
	10	(923)	(829)	(−94)	(183)	(137)	(−46)	(300)	(257)	(−43)	Calf had evidently suckled (replaced)
	11	681	756	+75	30	31	+1	0	4	+4	—
	12	765	698	−67	51	37	−14	0	3	+3	—
200*	13	739	734	−5	81	59	−22	50	119	+69	Calf may have suckled
	14	727	735	+26	22	50	+28	7	45	+38	—
	15	755	723	−32	14	54	+40	15	76	+61	—
	16	773	770	−3	41	43	+2	6	54	+48	—
400*	17	711	783	+72	58	92	+34	—	—	—	—
	18	730	809	+79	0	64	+64	5	59	+54	—
	19	750	873	+123	58	52	−6	0	82	+82	—
	20	832	860	+34	47	56	+9	0	82	+82	—
Range for five normally reared calves	—	—	860 to 1336	—	—	91 to 354	—	—	114 to 600	—	—

* See Aschaffenburg *et al.* (1949), p. 197.

turbidity test gave positive results with the serum of calves that had received relatively small quantities of colostral proteins, it has since been used to good purpose in eliminating from experiments newborn calves that had probably suckled their dams before being collected from the farms.

Special observations

(1) Data have been included in Table 1 for two calves (nos. 1 and 10) that had evidently received colostrum before they were collected.

(2) With two other calves (nos. 7 and 13), the initial strongly positive turbidity tests, supported by unusually high euglobulin nitrogen values, also suggest that these animals had managed to suckle.

(3) Quite normal results were obtained for the initial sample from the only survivor of the group of calves deprived of colostrum (no. 3), but marked increases in total and euglobulin nitrogen and in globulin turbidity were found with the 4th day's sample. This suggests that the calf had, in fact, obtained colostrum. It can only be assumed that it had suckled such a short time before the first blood sample was taken, that no effective transfer of colostral proteins into the blood stream had yet occurred.

SUMMARY

1. When newborn Shorthorn bull calves received an initial allowance of the non-fatty fraction of colostrum (Aschaffenburg *et al.* 1949) not exceeding 400 ml., the blood-serum euglobulin did not increase consistently.

2. A simple turbidity test for serum with *c.* 0·7 mM-zinc-sulphate solution was developed. It proved sensitive to the transfer of 'immune lactoglobulins' from as little as 200 ml. of the non-fatty fraction of colostrum.

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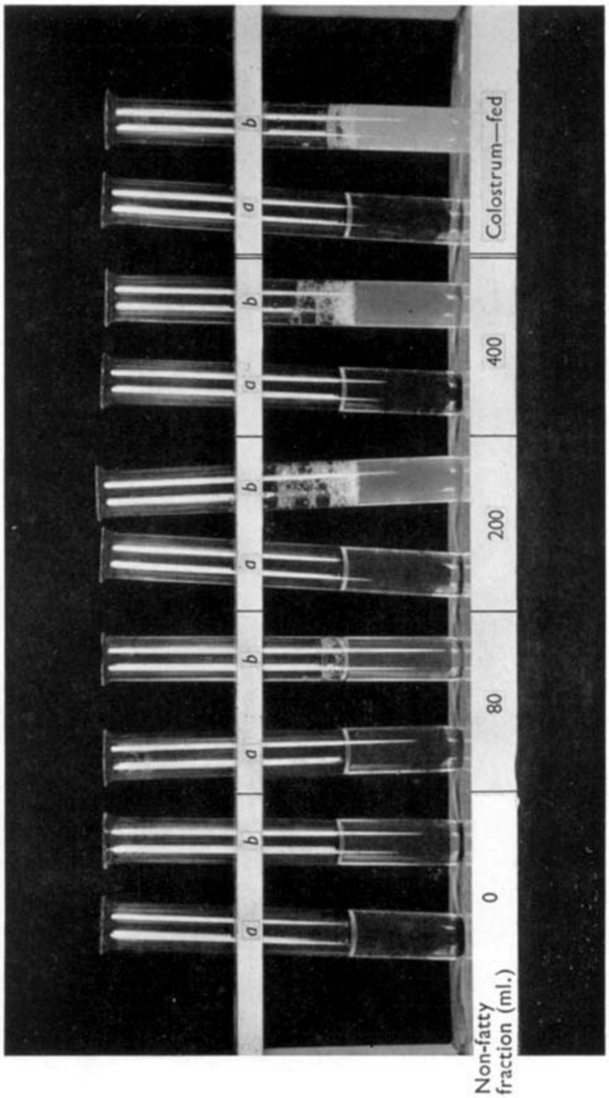
EXPLANATION OF PLATE

Pl. 1. Typical results with the serum of calves that had been given different quantities of the non-fatty fraction of colostrum, and of a calf that had suckled its dam. *a* = serum obtained before the first feed; *b* = serum obtained on the 4th day of life.


Non-fatty fraction (ml.)
0
80
200
400
Colostrum—fed
a
b