in erythrocyte membrane constituents had set in one to two weeks prior to the significant decrease ($P<0.01$ to $P<0.001$) in the resistance of sheep erythrocytes to osmotic lysis (0.77 ± 0.02 to 0.82 ± 0.02 initiation; 0.54 ± 0.05 to 0.65 ± 0.02 completion). In chronically infected lambs, the various erythrocyte membrane constituents were restored to near-normal (cholesterol, 1.28 ± 0.12 to 1.32 ± 0.11; cholesterol to phospholipid ratio, 0.93 ± 0.09 to 1.00 ± 0.08; acetylcholinesterase activity, 17.90 ± 1.45 to 18.15 ± 1.25). The erythrocytes of these animals continued to remain osmotically more fragile (0.70 ± 0.02 to 0.74 ± 0.02 initiation; 0.50 ± 0.02 to 0.56 ± 0.03 completion) than those of uninfected controls (0.67 ± 0.01 to 0.70 ± 0.03 initiation; 0.46 ± 0.01 to 0.48 ± 0.01 completion). However, the differences were not significant.

It is interesting to observe that the increase in erythrocyte fragility is preceded by a decrease in various membrane constituents during acute course of infection. Further, the alterations in these constituents coincide with the development of the nematode parasite to adult stage and its establishment in the air passages of the host, causing progressively increased interference with free flow of air and gaseous exchange in the lungs. In acute infection, the parasite is known to cause hypoxia and hypoxaemia[7], whose degree is associated with the extent of increase in osmotic fragility of the erythrocytes in this host–parasite system[8]. This is further supported by the present observation of restoration of osmotic fragility to near normal level in chronically ill animals.

Altered susceptibility of erythrocytes to osmatic lysis has been reported in disease and toxic conditions[3], besides structural defects and damage to erythrocyte membranes[9]. The decrease in membrane cholesterol, cholesterol to phospholipid ratio and acetylcholinesterase activity, seem to be the direct effects of hypoxic injury suffered by the sheep erythrocytes during acute infection. This is corroborated by the restoration to near-normal of these membrane constituents and erythrocytic fragility in chronic stage of infection (27th week PI onwards), when the infected host is relieved to a large extent from the hypoxic effects of the disease through various host compensatory mechanisms, besides worm expulsion from the lungs by acquired immune mechanism.

This happens to be the first study of erythrocyte membranes in helminth infections of man or animals.

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Some observations on goats following administration of *Leptospira interrogans* serovar *hardjo*

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***Leptospira interrogans* serovar *hardjo* cells were inoculated in two goats and bacteriological, serological and biochemical observations were made at various intervals. The goats exhibited a biphasic fever curve, the increase in temperature (104–105°F) being the first observed at 9th or 10th day post-inoculation (DPI) lasting up to 20th or 21st DPI. The second curve was observed between 22nd or 25th DPI and 32nd DPI. No other clinical sign was observed in any goat. During pyrexic phase leptospires were visible microscopically in the blood. However, attemps to culture the organisms, either from the blood or urine or tissue collected during necropsy failed. Antibodies against the *hardjo* cells were detected in goats during the initial pyrexic curve employing microscopic agglutination test (MAT) (at 14th DPI) and indirect haemagglutination test (IHA) (at 7th DPI). MAT titres in the goats remained more or less unchanged whereas the IHA titres declined. Biochemical studies carried out using sera of infected goats indicated an increase in liver enzymes namely alanine aminotransferase and aspartate aminotransferase and elevated bilirubin and cholesterol levels, suggesting liver damage. Albumin concentration in goats decreased with increase in globulin concentration.**

LEPTOSPIRA INTERROGANS serovar *hardjo* is an important pathogen of almost all the farm animals. The characteristic clinical features which develop in animals following the infection with this organism are abortion, still birth, infertility and mastitis 1,2. In goats, however, the only clinical sign which has been reported to develop following the administration of *hardjo* strains is a rise in rectal temperature[1]. No other information on infection in goats is available.

In the present study efforts were made to study biochemical changes in goats inoculated with *L. interrogans* serovar *hardjo*. Bacteriological and serological observa-

tions were also made to study the progress of the disease.

Two female adult goats, Nos 1 & 2, were inoculated intramuscularly with 10 ml of normal saline solution containing 2×10^6 cells of *L. interrogans* serovar *hardjo* and blood collected at various days post-inoculation (DPI). A portion of the blood was inoculated in Ellinghauson and McCullough medium for culturing leptospires and the other for obtaining serum for serological study and blood chemistry. Rectal temperature of the animals was recorded daily.

Antibody titres in sera of infected goats were monitored[3] by employing the microscopic agglutination test (MAT) and an indirect haemagglutination test (IHA). For conducting MAT live *hardjo* cells were used whereas for IHA, lipopolysaccharides (LPS) from *hardjo* cells were extracted, according to Vinh *et al.*[4] and adsorbed on sheep red blood cells (SRBC) using glutaraldehyde[3]. These sensitized SRBC formed the antigen for IHA.

Serum constituents of the infected goats, viz. bilirubin were determined according to Ducci and Watson[5], whereas for estimating total protein and serum albumin the method of Greenburg[6] was followed. Serum globulin was determined by subtracting albumin concentration from total protein. The ratio of albumin and globulin (A:G) in serum was determined by dividing the per cent of albumin with globulin percentage. Serum cholesterol was determined by the method of Zlatkis *et al.*[7]

Serum transaminase, viz. aspartate amino transferase (AST) and serum glutamic pyruvic transaminase (ALT) were estimated colorimetrically following Reitman and Frankel[8].

The values obtained at different intervals were compared using Student's t test to study the significance of difference[9].

None of the goat developed any clinical sign during the study. Rectal temperature in goat No. 1 rose to 105°F 9th DPI and remained high until 20th DPI. In goat No. 2 this rise was observed on the 10th day and lasted until 21st DPI. This fever response was followed by another pyrexic curve starting at 22nd DPI in goat No. 1 and 25th DPI in the other goat. By 32nd DPI the temperature in both the animals had returned to normal. Attempts to isolate leptospires from urine and blood of the goats collected at various intervals were not successful. Direct microscopic testing of these sample also did not yield leptospires except once in blood during 10th and 15th DPI when the goats were experiencing high fever. At 68th DPI the goats were sacrificed and the organs examined for gross pathological lesions and the presence of leptospira organisms. No pathological lesions were visible in any of the organs. Leptospira organisms could not be isolated from any of the organs even up to 3 months of incubation of inoculated media.

MAT and IHA titres in both goats were nil during the beginning of the experiment. At 7th DPI MAT titres in both the goats were 1:80 which rose to 1:160 only in goat No. 1 at 14th DPI and remained unchanged throughout the study. The other goat showed a titre of 1:60 at 21st DPI which also did not change until 63rd DPI when the study was terminated.

IHA titres in both goats were 1:128 at 7th DPI which declined to 1:32 at 14th DPI and remained unchanged up to 21st DPI. A further decline was observed at 28th DPI (1:16). The titres in goats did not decline further when tested at 42nd DPI and 63rd DPI.

Mean values obtained in goats on various biochemical parameters are given in Table 1. AST and ALT activities in goats increased after the inoculation of leptospira culture. Maximum increase was recorded at 28th DPI. At 63rd DPI the ALT activity returned to normal value but AST continued to be high ($P < 0.005$). Bilirubin in animals also increased following inoculation. The total protein in animals did not change whereas albumin showed significant decrease at 21st and 28th DPI. On the other hand, the globulin in goats increased and remained significantly higher throughout the study. Similarly serum cholesterol level in goats increased after inoculation and remained high during the study period.

The administration of *hardjo* in goats resulted in the production of biphasic fever curve without any other symptom being noticed. No gross pathological lesion of any kind was visible on post-mortem. Thus the administration of *hardjo* resulted in a mild reaction possibly

Table 1. Mean changes in biochemical parameters observed in goats at different days.

Days post-inoculation	ALT (RF Units/ml)	AST (RF Units/ml)	Serum bilirubin (mg/dl)	Total Protein (gm/dl)	Albumin(A) (gm/dl)	Globulin(G) (gm/dl)	A:G ratio	Serum Cholestrol (mg/dl)
0	20.00 ± 1.4	51.50 ± 6.3	0.29 ± 0.02	6.15 ± 1.24	3.18 ± 0.004	2.97 ± 0.006	1.07 ± 0.01	107.85 ± 3.32
14	33.00 ± 4.24^a	92.50 ± 2.12^c	0.49 ± 3.0	6.34 ± 0.11	2.84 ± 0.20^a	3.50 ± 0.03^a	0.81 ± 0.07^a	190.8 ± 4.3^b
21	40.9 ± 2.12^b	139.0 ± 2.82^a	0.41 ± 1.4	6.20 ± 0.40	2.46 ± 0.12^b	3.74 ± 0.20^a	0.66 ± 0.01^b	201.22 ± 4.2^b
28	41.5 ± 0.70^b	165.5 ± 6.26^b	0.57 ± 5.2^c	6.18 ± 0.06	2.58 ± 0.02^b	3.6 ± 0.08^a	0.71 ± 0.02^a	206.8 ± 5.1^b
42	35.5 ± 3.5^a	168 ± 6.36^b	0.70 ± 5.3^c	6.52 ± 0.4	2.80 ± 0.15	3.72 ± 0.32^d	0.75 ± 0.02^a	206.3 ± 4.7^b
63	25.5 ± 0.7	150 ± 11.3^c	0.60 ± 1.34^c	6.10 ± 0.10	2.66 ± 0.07	3.44 ± 0.03^d	$0.77 \pm 0.0c^a$	180.04 ± 7.6^a

[a] = Significant change from day 0($P < 0.01$).
[b] = $P < 0.001$.
[c] = $P < 0.005$.
[d] = $P < 0.025$.

due to leptospiraemia, as reported earlier in cattle[10]. The occurrence of a biphasic fever curve was interesting. This may be due to reseeding of organisms in the blood from liver, spleen, etc. where organisms could have been lodged following the initial leptospiraemia phase. This requires further investigation as the organisms could not be demonstrated in these organs collected during post-mortem, either culturally or by direct microscopy. It is possible that the organisms did localize initially but were soon cleared as a result of the development of immune response as indicated by the presence of MAT and IHA titres in animals and the increase in total globulin contents.

The immune response elicited in animals following the inoculation of *hardjo* cells was milder in nature. MAT titres in these animals developed slowly and did not change appreciably. However, the titres as detected by IHA developed quickly and then declined. These observations suggested that in fact two different antibody classes were detected by these tests in spite of the fact that in MAT and IHA, antibodies directed against lipopolysaccharide antigen were supposedly responsible for agglutination. It appears that a change in location and the native form of the antigen were responsible for such a difference.

Table 1 reveals an increase in serum cholesterol and bilirubin at various DPI. The former could be due to decreased excretary function of the liver[11]. It is postulated that the organisms caused damage to hepatic parenchyma resulting in a decrease in the volatile and higher fatty acid uptake by liver for glucogenesis which entered the circulation to cause hypercholesterolemia. Increase in the bilirubin concentration was again suggestive of the damage of liver parenchyma which caused a decrease in excretion of bilirubin. Abnormally high values of AST and ALT were observed from 14th DPI onward. An increase in the liver enzymes due to hepatic damage has been observed in different farm and laboratory animals[12,13]. A two to three-fold increase in AST and ALT values after *hardjo* administration indicated a significant damage to hepatic cells. All these findings were thus indicative of liver damage. Virulent leptospires have predilection for parenchymatous organs such as liver[14], spleen, kidney, etc. subsequently causing local damage. The present study suggests that in goats liver is affected. However, the use of several strains is suggested to see whether other organs are also involved.

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Insulin tolerance in lean and obese hens

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Insulin caused significant decline in blood glucose levels within half an hour after injection in breeder hens. Rate of decline, however, was much lower in heavier birds than in lighter ones. Plasma cholesterol and free fatty acid levels did not differ significantly. Plasma VLDL+LDL levels were altered significantly, but the pattern of change among high-, medium- and low-body-weight birds was opposite to that of blood glucose. VLDL+LDL levels were highest in heavier birds and remained elevated longer than in lighter birds. The response of lean and obese individuals to insulin appears to be different and could be one of the reasons for obesity.

THE mechanisms, involved in the abnormal insulin secretory dynamics, associated with obesity are unclear. Although insulin resistance is associated with obesity, it is not clear whether obesity regulates insulin levels or whether insulin levels lead to obesity[1].

Glucose–insulin imbalance was earlier considered to be one of the possible reasons for obesity in high body fat depositing individuals[2]. Lower levels of plasma glucose in high abdominal fat depositing individuals have been observed[3]. As in humans, over 90% of the total body lipogenesis occurs in the avian liver[4]. An experiment was conducted to study the effect of exogenous insulin injection on carbohydrate–lipid interactions in high and low body weight birds used as model for humans.

Thirty-six adult broiler breeder hens were divided into three groups of 12 birds each on the basis of their body weight. The average body weights in the high, medium and low body weight groups were 3.76 ± 0.75,